

24 September 1981

Chance for a change in universities

The disappearance of Mr Mark Carlisle, until two weeks ago the Secretary of State for Education and Science in the British Government, is not widely lamented. In higher education, he will be remembered chiefly for having brought about the most serious crisis in British universities since the seventeenth century. Mr Carlisle's political colleagues, even those who consider that the universities need to be taught a lesson, or forced by economic hardship to change, may well be embarrassed that Mr Carlisle has left them with the problems that will arise when the universities dutifully comply with the wishes of the University Grants Committee by firing members of the teaching staffs, thereby landing the government two or three years from now with an unquantifiable claim for compensation from academics able to show that their contracts are not compatible with summary dismissal. But Mr Carlisle's departure will not send people dancing to their lecture rooms as the new academic year begins. For Sir Keith Joseph, Mr Carlisle's successor, has an ambiguous reputation. Nobody can be sure whether he will be willing to repair the damage that Mr Carlisle has wrought.

Even so, the universities should not neglect the opportunity presented by the arrival of a new secretary of state. Fortunately, the universities are able to judge (from Sir Keith's performance in the past two years at the Department of Industry) what kinds of arguments are likely to sway him. Sir Keith is theoretically a hard-line conservative — a monetarist believing that market forces are the best arbiters of how resources should be allocated. In practice, however, Sir Keith is also something of a softie. Faced with demands from nationalized industries such as British Steel or British Airways that they should be allowed to operate at a loss, Sir Keith's heart has almost always melted and he has written them cheques (drawn on the British taxpayers' account). If the universities are to win some relief from the 8.5 per cent reduction (over three years) in the direct subvention of universities, together with some redistribution of the hardship caused by the random consequences of the withdrawal of overseas students from the British university system, they had better put to him an appeal based on a blend of judicious argument and sob-story. This is how the case should be put.

First, the market does not apply to higher education. The universities have been told, by the University Grants Committee, that they must reduce the numbers of students that they teach, but the numbers are arbitrary and nothing comparable has been said about polytechnics because the government has been late in setting up a mechanism for their control. The result is that potential students have been flocking to the polytechnics, fearing that the universities would have no room. The result may be that the public purse may not save, in this first disastrous year, even the mandatory subvention of student maintenance that it pays through local authorities. At the same time, students will finish up in places they would not have chosen. That, to some extent, has always been the case, given the grants committee's habitual guidance on student numbers. So maybe the time has come to restore competition to the market for higher education by requiring that all institutions of higher education — polytechnics as well as universities — should charge even students from the United Kingdom full economic fees (defined by their own estimates of what these are, not calculated in Whitehall), using the cash thus liberated (say £1,000 million a year) to make sure that able students are not denied a chance of a decent education. In short, the time may have come for making a start on financial independence of the universities and polytechnics from govern-

ment, and for a recognition that its social obligation is towards students and not academics.

Second, the universities must argue that the errors of the past months are based almost entirely on ignorance. People outside higher education constantly complain that the universities cannot change quickly enough, and it is true that some of them are incapable of changing at all except when forced to do so. But it is folly to suppose that individual institutions can change as rapidly as the University Grants Committee has reluctantly decreed that some must. To suppose that a university can contract by, say, a fifth in three years is to suppose that the interests of many of its students (following three-year courses) can be neglected. The consequences of the random distribution of hardship through the system must, at the same time, ensure that academic teachers suffer arbitrarily, and that the institutions themselves are often robbed of the skills they can least spare. This is the sob-story of which Sir Keith must be told. It could melt all but the hardest heart. For what is now in prospect is that most institutions of higher education will be permanently damaged.

Will the Committee of Vice-Chancellors, on whom the responsibility falls, have the courage to make these arguments? Only time will tell. The universities as a whole would prefer a return to the happy days of old, when the University Grants Committee made sensible recommendations about the allocation of resources and the government paid the bills. A substantial shift towards the concept of the financially autonomous university, dependent for its survival on its ability to attract students and research funds, would be unwelcome to those academics who would be offended that the principle of state-subsidized higher education had been breached. And academic life would become more tedious without a familiar grants committee to ensure stability. Whatever the ideal, however, the immediate need is survival. And beggars are not well placed to choose how they survive.

A book for burning?

Books rightly command respect, even affection. They are the products of sustained creative work, and even if their authors' ambitions are not fully realized, find a niche somewhere as pebbles on the beach of scholarship and literature. And even bad books should not be burned; works such as *Mein Kampf* have become historical documents for those concerned with the pathology of politics. But what is to be made of Dr Rupert Sheldrake's book *A New Science of Life*, published in the summer (Blond and Briggs, London, 1981)? This infuriating tract has been widely hailed by the newspapers and popular science magazines as the "answer" to materialistic science, and is now well on the way to being a point of reference for the motley crew of creationists, anti-reductionists, neo-Lamarckians and the rest. The author, by training a biochemist and by demonstration a knowledgeable man, is, however, misguided. His book is the best candidate for burning there has been for many years.

The argument is simple. If physicists cannot confidently calculate crystal structures or molecular configurations *ab initio* from a simple knowledge of their ingredient atoms, surely it is unthinkable that molecular biologists will be able accurately to relate the genetic structure of an organism to the successive phenotypes that arise during the course of its development? And the difficulty is not merely computational. Do not the cells of arms and legs contain exactly the same DNA, Sheldrake asks?



And how can it be that the very similar genomes of human beings and chimpanzees give rise to very different phenotypes when, in *Drosophila*, much larger differences of genotype specify flies that are very similar in form? Sheldrake's way of dealing with these and other observations is to borrow from embryology the vague notion of the morphogenetic field, the name given to the largely unidentified influences that give shape to a developing organism, and then to exalt this into a kind of universal agency. All aggregations of matter, animate or otherwise, are supposed to be exposed to a great variety of morphogenetic fields which fill the whole of space and which travel forwards in time. The effects of these morphogenetic fields (which are otherwise not described) are to ensure that particular aggregations of matter take on the form assumed by similar aggregations of matter elsewhere or at some earlier time. This is the "hypothesis of formative causation" — the subtitle of the book. The inheritance of acquired characteristics is at a stroke explained. Jung's collective unconscious is made inevitable. Instinctive animal behaviour becomes a non-problem, for there will be a morphogenetic field that ensures that each set of neurones in each central nervous system is put into an appropriate correspondence.

This argument would not be taken seriously were it not that others have done so. As things are, however, Sheldrake's book is a splendid illustration of the widespread public misconception of what science is about. In reality, Sheldrake's argument is in no sense a scientific argument but is an exercise in pseudo-science. Preposterously, he claims that his hypothesis can be tested — that it is falsifiable in Popper's sense — and indeed the text includes half a dozen proposals for experiments that might be carried out to verify that the forms of aggregations of matter are indeed moulded by the hypothetical morphogenetic fields that are supposed to pervade everything. These experiments have in common the attributes of being time-consuming, inconclusive in the sense that it will always be possible to postulate yet another morphogenetic field to account for some awkwardly inconclusive result and impractical in the sense that no self-respecting grant-making agency will take the proposals seriously. This, however, is not the most serious objection to Sheldrake's attempt to endow his argument with an appearance of orthodoxy. The more serious objection to his argument is that it says nothing of any kind about the nature and origin of the crucial morphogenetic fields and contains no proposals for investigating the means by which they are propagated. Many readers will be left with the impression that Sheldrake has succeeded in finding a place for magic within scientific discussion — and this, indeed, may have been a part of the objective of writing such a book. But hypotheses can be dignified as theories only if all aspects of them can be tested. Sheldrake's hypothesis is no better than the hypothesis that a person equipped with a water-divining rod is able to detect subterranean water as a consequence of some intervening "field" generated by the presence of water, and his proposals for experimental tests no better than the argument that since water-diviners succeed in making money, there must be something in the theory.

It is naturally distressing that these plain truths are not more widely understood. It is also a misfortune that the public expectation of what science can accomplish, now coloured by Dr Sheldrake's argument, should so conspicuously lack patience. Sheldrake's argument takes off from his catalogue of the ways in which the molecular biologists, no doubt the shock-troops of the reductionists, have so far been unable to calculate the phenotype of a single organism from a knowledge of its genotype. But so what? Have not the past twenty years shown clearly enough that molecular explanations of biological phenomena are, contrary to some earlier expectations, possible and powerful? And who says that molecular biology must be counted as a failure for as long as embryology is not a branch of mathematics? Dr Sheldrake, whose background should have enabled him to know better, has done a great disservice by helping to give currency to these misconceptions and false expectations. His book should not be burned (nor even confined to closed shelves in libraries) but, rather, put firmly in its place among the literature of intellectual aberrations.

Back to gold standard?

President Ronald Reagan's budgetary problems, not very different from those afflicting other governments in industrialized states, have nevertheless provoked a distinctively American and exceedingly curious response — the notion that present economic troubles might be made to go away by putting back the clock to 1971 and returning to the gold standard that was then abandoned. The proposal has surfaced on several occasions in the past few weeks, chiefly by means of hints or asides in the speeches of conservative economists and politicians. Last week, in a speech at Denver, Vice-President George Bush went so far as to say that this proposal should be seriously considered. He is clearly unaware that this stratagem for dealing with the economic problems of the United States is about as sensible as the proposal that fever should be treated by the immersion of the patient in iced water.

None of this implies that the problems of the United States economy can be allowed to solve themselves. On the contrary, it has become clear in the past few weeks that radical measures of some kind have become essential. The essence of the problem is that, at a time of rapid inflation, the Administration is planning to spend more than its revenue. Official estimates are that in the present financial year, the deficit will approach \$50,000 million, but both the Congressional Budget Office and the financial markets clearly believe that this estimate will be exceeded. So the Administration is hurriedly embarked on a process all too familiar in countries such as Britain — making last-minute adjustments to spending plans for the coming year and those that will follow. Mr Reagan now plans to reduce next year's spending by \$17,000 million, with larger cuts to follow in succeeding years, but the announcement of these plans has not steadied nerves in Wall Street nor brought interest rates (running at 20 per cent) down to the point at which industry will be encouraged to invest in the reinvigoration of the United States economy. The dilemma is depressingly familiar.

There is no doubt that a return to the gold standard would superficially be beneficial. Once it has been agreed that those who hold dollar bills may turn them into gold whenever they choose to do so, the capacity of governments to outspend their revenues is strictly limited by their capacity to find more gold to stockpile. Breaking the rules of sound money management becomes strictly impossible. But there is no reason why governments should put themselves in such a straitjacket simply with the objective of being forced by external circumstances to behave in a way they recognize to be desirable when they could achieve the same result by pure reason — by the exercise of self-restraint — and need not then have to squander resources on acquiring gold. Moreover, the disciplines of the gold standard can be needlessly cramping. Even monetarists agree that there may be circumstances, at the end of a long slump for instance, when governments would be wise to spend more than they raise in the form of taxes. To be tied in monetary policy to an artificial standard is not merely a confession of failure but potentially disastrous.

These are the reasons why Vice-President George Bush should not continue in public to speculate about the possibility that Fort Knox might be restocked with gold. If the Administration must have some crutch on which to lean in the months immediately ahead, it would be better advised to link the value of its currency to some quite different external standard, perhaps some short-lived exotic radioactive isotope whose permanent influence on the economy would be small. The trouble is that those holding dollars would be unwilling to trade their paper currency for such a material. In the circumstances, the Administration probably has no choice but to rely on the restraints that exist at present, the chief of which is the unwillingness of Congress to sanction the promised tax reduction, the planned increase of military expenditure and the reduction of welfare expenditure. That is why the present crisis in the United States economy has become a political as well as an economic embarrassment. With such obdurate critics, the Administration has no need of Fort Knox.

NASA space science prospects still grim

ESA forced to go solo for the Sun

Washington

European space officials have failed to dissuade the United States from making a substantial withdrawal from the International Solar Polar Mission (ISPM) planned as a joint two-vehicle mission between the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA).

Faced with further severe cuts in space science programmes as part of the Reagan Administration's attempts to balance the federal budget, NASA has decided not to ask for any provision in the 1983 budget for the spacecraft which NASA was to contribute to the joint mission.

As a result of this decision, which ESA Director-General Erik Quistgaard was informed of by NASA administrator James M. Beggs two weeks ago, ESA has reluctantly accepted that it will have to fly its own space vehicle solo. Scientists will not now be able to obtain the hoped-for three-dimensional view of events on and around the Sun's surface. ESA's space advisory committee met in France last week to decide how it should proceed with a project on which over \$100 million of European research funds has already been spent.

Details of the budget for the 1983 financial year, beginning on 1 October 1982, have not yet been presented to Congress, but it is thought that the Office of Management and Budget has told the agency to plan for a total budget of about \$5,000 million, \$500 million less than the 1982 figure — and, allowing for inflation, a sum that would represent a 20 per cent decrease in funding for space research and development.

A political desire to protect the space shuttle from serious cuts would mean most cuts falling on the space science and space applications areas. With other programmes now under threat, including in particular the Galileo mission to send an orbiter and probe to Jupiter in 1985 as well as the whole of NASA's Solar System exploration division, the prospects of reviving a substantial US involvement in ISPM are now almost non-existent.

The Reagan Administration announced in March that, as part of the reduction in the 1982 budget, it proposed withdrawing the US spacecraft from the joint mission and reducing NASA's contribution to support for US scientific experiments on board the European craft. NASA will, however, be providing launch, tracking and data retrieval facilities as arranged.

The decision caused European space officials to protest that it was unilateral abrogation of the agreement between the two agencies which would not only reduce the scientific value of the mission, but also jeopardize the chances of future collaboration in space science projects.

In an attempt to salvage a dual-spacecraft mission, ESA had suggested that NASA could reduce its costs by ordering a duplicate version of the European spacecraft, to be built by the same Star consortium with the German company Dornier as prime contractor. The cost would have been \$40 million (with ESA underwriting any increase), rather than the expected \$100 million for the vehicle which NASA was to have had built by TRW Inc.

A mission with two identical vehicles, however, would have eliminated the coronagraph and the X-ray/X-ray-UV imaging which the NASA design would have provided. A report prepared for the Congressional Appropriations Committee by an *ad hoc* panel of the National Academy of Sciences concluded that this option was less attractive than flying the ESA craft and a cheaper NASA spacecraft without the despun platform or imaging instrumentation; and that even the increased scientific return of the latter option was "not commensurate" with the increase in cost over flying the European spacecraft on its own.

David Dickson
Philip Campbell adds: Following a meeting last week, ESA's scientific advisory committee has requested the agency's

board of national delegates to endorse the continuation of the truncated International Solar Polar Mission. NASA's decision not to continue with the development of its spacecraft reduces only some of the uncertainty surrounding the project. ESA's spacecraft (which will carry nine experiments including six with principal investigators from the United States) depends on NASA's upgraded Centaur vehicle for its launch from the space shuttle. But the development of the Centaur launcher, also to be used in NASA's planned Galileo mission to Jupiter, is itself in doubt because the Galileo mission is under scrutiny as a result of budgetary pressures.

If the continuation of the European half of the solar polar mission is endorsed, ESA will continue to develop its spacecraft for a 1983 launch, despite the fact that NASA is working towards a launch in 1986. ESA's shorter timetable will minimize costs and, although the agency is prepared to put its vehicle into cold storage, it hopes to persuade NASA to bring forward the launch date, Centaur development permitting.

The two spacecraft were designed with only four experiments in common. ESA does not have the funds to rescue other experiments that were unique to the NASA vehicle. Although no specific "reprisals" have been announced, it is expected that NASA's decision not to continue with the solar polar mission will severely damage prospects for future collaboration in space between Europe and the United States. □

Racy reforms due for French research

The missionary zeal of the French government to reform the nation, preferably tomorrow, is not lost at the Ministry of Research and Technology. A few days ago the principal research agencies, such as the Centre National de la Recherche Scientifique (CNRS), were officially given three weeks (until 1 October) to make a claim for a place in a four-year programme of research, encompassing most of the billion-pound budget of the ministry.

At the same time, the minister announced that he was embarked on a "deep reform" of the CNRS, and won a 50 per cent increase in the budget of ANVAR, the Agence Nationale pour la Valorisation de la Recherche, which supports fledgling innovation in French industry. (ANVAR could do with more support it seems — recent figures showed new French patents last year amounting to only 1.7 per cent of the world total, compared with 4 per cent in 1975.)

Admittedly, the research agencies need only submit outline budgets, staff requirements and research programmes — but the timetable gives little room for manoeuvre. Then on 7 October the regional meetings of

a "National Colloquium" on science and technology will begin, with the position papers of the agencies in hand. The full colloquium is due in Paris in mid-January, and, according to the minister, Jean-Pierre Chevènement, that colloquium will finally decide the lines of the four-year *loi programme* which will define France's research commitments to 1985. (Previously the rigid form of a *loi programme* — which fixes budgets, staff and strategy by law for a long period — has been used only by the Ministry of Defence; Chevènement hopes to apply it to research.)

However, it is unlikely that such a broad forum will do other than given Chevènement plenty of room to define his own programme, taking account of the political factions which will emerge out of the scramble of the next few months.

Chevènement has already started to undo the reforms of CNRS instituted in 1979 by the minister of the universities of the previous government, Madame Saunier-Seïté. She saw fit to remove the right of technicians and administrators to be elected to the Comité National — a kind of scientists' "parliament" of CNRS, unique among Western research agencies. But the

Comité has important powers — such as over whether unsuccessful laboratories should be closed, or new ones opened — and the technicians' and administrators' union, affiliated to the communist-led CGT, has been campaigning ever since for its voice to be heard again in that forum.

Chevènement agrees, and hasty changes are being made in order to allow technicians and administrators to attend the autumn meeting of the Comité. Beyond that, there must be a reform of CNRS statutes, which will probably wait for the National Colloquium; but the reform will be thorough, and could even involve the abandonment of the present two-tier directorship of CNRS, which has a scientific president and an executive director with overlapping responsibilities.

In a further move, the Ministry of Research and Technology has begun to appoint its own staff. They will be organized in *missions d'orientation*, concerned with the various goals of Chevènement's policy. Directors have been named for four. "Renewable energy and conservation" will be headed by Philippe Chartier, an expert in biomass from the Institut National de la Recherche Agronomique; "biotechnology" by Pierre Douzou, an ex-professor at the Museum of Natural History; "electronics" by Abel Farnoux, a television-tube manufacturer; and "employment and the conditions of work" (a matter of great concern to Chevènement, and the government as a whole) by an ex-cabinet maker and present assistant lecturer on working conditions at the University of Paris IV, Albert Detraz.

Robert Walgate

British universities

Job losses inevitable

News is emerging from British universities on how they plan to contract along the lines indicated by the University Grants Committee early in August. Not surprisingly, those universities singled out for the largest cuts in their recurrent grants over the next three years are among the first to react. Some estimate that they need to lose between 50 and 150 academic posts and up to 450 non-academic posts. Although all concerned hope that posts can be lost through early retirement and voluntary redundancy, it seems that compulsory redundancies are inevitable.

The biggest problem for universities singled out for cuts in income of more than 20 per cent is that action needs to be taken quickly. The senate of Brunel University, for example, is this week considering a report from a working party which recommends losing 110 non-academic posts, preferably by the end of this year, and 50 academic posts by April 1982.

The University of Aston in Birmingham, which says that it must do away with 150 academic and 450 non-academic posts to comply with the grants committee's wishes, has a

London to respond with more committees

The University of London, which has been agonizing over its future organization for some time, has now set up four subject review committees to advise on the resource allocation most likely to maintain high academic standards. This move comes just a few weeks after the university court told the constituent colleges how much money they will have to spend in the current academic year and shortly before the Committee on Academic Organisation, under its chairman Sir Peter Swinnerton-Dyer, is due to issue its final report on how the university should be re-constructed.

The committees, which have been established too late to have much bearing on this year's allocations, have nevertheless been charged with the task of reporting quickly. The hope is that recommendations made early in 1982 can

be used in shaping the university to its reduced grant in future years.

The move may also provide alternatives to the recommendations made by the Swinnerton-Dyer committee in its interim report. Those included the unpopular suggestion that Chelsea College be closed and that some of the smaller colleges should merge.

Four independent chairmen have been appointed to the committees whose detailed membership will be announced later. The committees are on physical sciences, under the chairmanship of Sir Sam Edwards from the University of Cambridge; biological sciences, under Sir James Beament of the University of Cambridge; languages under Professor J. Cruickshank from the University of Sussex; and social studies under Sir Alec Cairncross of the University of Oxford.

Judy Redfearn

similar problem. Its decision last week to inform the Department of Employment that 95 staff will be redundant within a month aroused strong opposition from the Association of University Teachers, the academics' trade union, which threatened to take the university to court at the first hint of compulsory redundancy. The university administration claims that these redundancies are for non-academic staff who have already opted to take early



William Shelton, MP for Streatham in London, who has replaced Neil Macfarlane as Britain's Under-Secretary responsible for science in the Department of Education and Science

retirement or voluntary redundancy with compensation of up to nearly three times annual salary, but compulsory redundancies seem inevitable sooner or later.

Other universities, however, are taking it a bit more slowly. The University of Bradford plans to meet its 30 per cent drop in income by 1983–84 by losing between 150 and 180 academic staff over three years. Consultations between the senate and heads of departments should result in a strategy by December.

Universities with more modest cuts in income are also taking more time, some of

them hoping to get by without compulsory redundancy. The University of Cambridge plans to shed 100 academic posts by lowering the compulsory retirement age from 67 to 65 years and by filling only essential posts that fall vacant. It plans to make further savings by, amongst other things, abolishing a student travel fund, curtailing sabbatical leave and abolishing examiners' fees. And at the University of Bristol, where it has been suggested that academics take a cut in salary, no decision has yet been taken. Much still needs to be resolved, however, before the future shape of the British university system becomes clear. During the next few weeks, universities will be presenting the grants committee with detailed plans, some of which will go against the committee's original advice. The University of Bradford, for example, would prefer to cut its physics and chemistry departments rather than biology. And the University of Leeds plans to make cuts across the board before resorting to selective cuts between departments.

Some universities are clinging to one last hope: that Sir Keith Joseph, new Secretary of State for Education and his new Under-Secretaries, William Waldegrave for higher education and William Shelton for science, can be persuaded to give the universities more time.

Judy Redfearn

Polish universities

Poised to strike

Solidarity members at Krakow's Jagiellonian University and the nearby Mining and Metallurgical Academy (AGH) have declared a state of "strike readiness" in protest against unilateral amendments by the Ministry of Science, Higher Education and Technology to the new draft law on higher education. Many

other universities and colleges throughout Poland have reiterated the anger of the academic community at what one protestor called the "outrageous" amendments. Both the new Independent Students' Association (NZS) and the "old" Socialist Union of Polish Students have issued protest statements, but because of the summer vacation, so far only the AGH students have had the chance to organize any action (their chapter of NZS has also declared "strike readiness"). NZS is still hoping for the situation to be resolved by negotiation. However, if necessary, it would take a referendum of its members on possible strike action as soon as the universities reconvene.

Dr Michal Pulawski, an assistant lecturer at the Jagiellonian University and chairman of its Solidarity chapter, who actually declared the alert, was interviewed on Polish radio last week. He again stressed that the Krakow academics hoped that commonsense would prevail and a strike would not be necessary. Nevertheless, he said, the proposed amendments — introduced in spite of the ministry's pledges to submit the bill to the Sejm (parliament) without further changes — would fundamentally alter the law. They relate, he said, to the financing of higher education, the right of higher educational institutions to teach a plurality of world views, and the ways of hiring and dismissing the employees of higher educational establishments. (The latter is a sensitive issue in Poland, where the academic purges of 1968 were a grim experience for the younger generation of intellectual activists.)

The academic community was particularly indignant, Pulawski said, about the way the changes had been introduced "without consultation and without discussion". Indeed, a back-up message from the Solidarity chapter at Krakow Polytechnic suggested that the ministry had deliberately tried to rush things through, granting the academics "barely one week" to make their stance known. Since strike readiness was declared at Jagiellonian University and AGH, talks have been resumed. Last week, meetings included a discussion of the controversial amendments between Professor Hieronim Kubiak, a secretary of the Central Committee of the Polish United Workers' Party, and the various university rectors, many of whom have been elected by the new democratic procedures which the amendments would, presumably, negate. During a lively encounter between the minister Dr Jerzy Nawrocki and journalists from the Club of Educational Publicists, Dr Nawrocki seemed to be preparing for a partial climb-down on the amendments. Nothing was final, he said; his ministry would "shortly" be considering all the views expressed, and would be starting a new round of discussions with the relevant trade unions and student organizations.

Vera Rich

Belgian nuclear energy Safety in question

Brussels

Serious failings in Belgium's nuclear safety policy have been brought to light in a new report. Commissioned by the Belgian government from the European Commission pool of nuclear experts, the report describes as "unhealthy" the manner in which the new plants Doel 3 and Tihange 2 are being constructed.

The report points out that in Belgium no safety survey is carried out on a nuclear power station until shortly before the plant goes active. By this time any outside expert advice is virtually useless, for either the plant cannot be altered, or at best only an uneasy compromise can be reached.

Ironically, the Belgian government had promised to tighten up nuclear power security following the Three Mile Island accident, but little has happened since then. The European Community experts now propose that Belgium should adopt the American procedural system whereby constructors of a nuclear power station must submit an initial safety study on which outside experts can suggest improvements. Then, before the plant goes into operation, a second study is carried out to demonstrate that the existing safety regulations and the new recommendations have been adhered to.

So far the Belgians have rejected this approach on the grounds that it takes too long and is too complicated.

The nuclear power industry's problems do not stop there. The International Atomic Energy Authority has already reprimanded Belgium for not engaging the 50 personnel it considers necessary to deal with matters of nuclear safety. The environmental group, Greenpeace, has been trying to stop vessels leaving Zeebrugge harbour with nuclear waste. And the 17 technicians at present working in Belgian plants are now threatening to go on strike because of a dispute over how the power stations are being run.

The workers are employed by a private company, Vincotte, which the Belgian socialist parties are trying to have replaced by a public authority. The Belgian government has been unable to reach agreement on this point and instead last month created two new departments in the Ministry of Work and Employment and the Ministry of Public Health, with responsibilities including the supervision of the work done by Vincotte. The money for this would come out of a special tax on the electricity produced.

This arrangement has so incensed the technicians at Vincotte that they have given notice of their intention to down tools. Furthermore, Vincotte's director has stated publicly that all power has now passed into the hands of those who do not have the necessary knowledge.

Jasper Becker

Change at OSTP

President Reagan's science adviser, Dr George A. Keyworth, has announced the reorganization of the Office of Science and Technology Policy (OSTP), which he heads. Two of the three previous associate directors under Dr Keyworth's predecessor, Dr Frank Press, will be staying on. Dr Benjamin Huberman, previously associate director for national security, space and international affairs, becomes deputy director of OSTP. And Dr Dennis Prager, who had been associate director for human resources and social and economic services, becomes assistant director for life science and institutional relations.



Keyworth — a busy man at home as well as abroad (see p. 251)

Four others have been appointed as assistant directors — John Marcum (energy and natural resources); Victor Reis (national security); Edward McGaffigan (international affairs); and Douglas Pewitt (general science). Major Thomas Johnson has been appointed a special assistant to Dr Keyworth, and is expected to be involved in issues concerning military technology.

David Dickson

US science policy

Room at the top

Washington

Members of the National Science Board (NSB) — the group of scientists appointed by the President to be responsible for the policies of the National Science Foundation (NSF) — have begun to debate whether they should play a more active role in national discussions on science and technology policy, urged on by their chairman, Dr Lewis Branscomb. Chief scientist at IBM, and at one time widely-tipped as President Carter's science adviser, Dr Branscomb has made little secret of his feeling that, rather than concern itself strictly with the research and education programmes supported by the foundation, the board should tackle broader policy issues such as the health of US technology and science and engineering education in general.

Other members of the board are less cer-

tain about expanding their role. Attending their monthly meeting in Washington last Friday, they were reluctant to declare themselves in favour of Dr Branscomb's ideas, preferring instead to refer them both to NSB committees and to officials of the foundation for further consideration.

Since their formation shortly after the Second World War, both board and foundation have been the focus of a broad debate over whether they should limit their interest to the basic research programmes for which they shared responsibility; or whether to occupy a more prominent position in national discussions on directions for US science and technology. Two factors in particular seem to have rekindled this debate. First, the apparent decision by President Reagan's science adviser, Dr George A. Keyworth III, not to resurrect the President's Science Advisory Board (PSAC) which performed this broader political role in the 1950s and 1960s until it was abolished by President Nixon in 1973. And second, the feeling that the Office of Science and Technology Policy (OSTP) is so closely tied to the executive responsibilities of the White House that it has little time to think about long-term strategies.

The discussion has been catalysed by a report being prepared for Dr Branscomb by Dr Philip Smith, previously an associate administrator of OSTP, and recently appointed executive officer to the National Academy of Sciences. Addressing the board members at last week's meeting, Dr Smith said that NSF in particular was in a

stronger position than in the past. "The NSF has become a big and mature organization which is respected throughout the world", Dr Smith said. "The time has come to think in terms of what the next steps are for the NSF as an institution; it is an opportunity to reform the foundation and to keep it alive."

Areas in which he suggested that a strong policy initiative might be of value included science and engineering education.

Among the more sceptical board members was its vice-chairman, Dr Herbert Doan, chairman of a venture capital company and a director of Dow Chemical. He suggested that broad policy statements were of little value without the authority to carry them out.

There was also reluctance by NSF director Dr John Slaughter to be swayed by Dr Branscomb's vision. He suggested that, rather than discussing the roles of the board and the foundation the emphasis should be on improving efficiency.

But Dr Branscomb was insistent. "I would urge the director [of NSF] not to underestimate the prestige of his office and the impact that he can have," he said. Dr Branscomb emphasized that he was not suggesting that NSB could, or should, take on the mantle of PSAC. But he supported Dr Smith's contention that many professional research societies and industrial research organizations "are looking for some entity on the Washington scene to provide leadership that has been lacking in recent years".

David Dickson

Cigarette smoking

Hope for habitués

Making cigarettes a more effective means of delivering their main pharmacological agent, nicotine, is the latest suggestion from the Tobacco Advisory Council for reducing the health risks of smoking.

A new publication, *Monograph on the Pharmacology and Toxicology of Nicotine*, sets out to examine the properties of nicotine itself rather than nicotine plus the various other components of cigarette smoke. The authors conclude that at the levels present in cigarette smoke nicotine is less toxic than many of the other smoke components, and that there is no conclusive evidence that nicotine contributes to the carcinogenic potential of cigarette smoke.

The trend towards lower tar and lower nicotine cigarettes does seem to be associated with a reduced death rate due to lung cancer. But many smokers subconsciously adjust their smoking patterns to restore their nicotine intake to their previous accustomed dose. So for them at



least, low-tar but medium-nicotine cigarettes might be the "cleanest" way of obtaining the dose of nicotine they feel they need, while minimizing the amounts of carcinogens and other toxins that they take in. So this new report should encourage cigarette manufacturers to continue looking for ways of increasing the amounts of nicotine that can be squeezed into a cigarette without increasing tar levels — a task that so far can only be done using rather expensive techniques.

Although reaffirming the notion that no cigarettes are safe, anti-smoking proponents are unlikely to take exception to the idea of finding a new balance of ingredients that may put confirmed smokers slightly less at risk than they are now. But the Tobacco Advisory Council monograph does include some points likely to be hotly disputed. For instance, although the medical profession is adamant in saying that pregnant women should not smoke, the authors state "... evidence is available showing that maternal smoking is not associated with the risk of developing fetal malformations". Strictly true that may be, but maternal smoking is associated with reduced fetal growth and low birth weight, increased spontaneous abortion, complications in pregnancy and increased perinatal mortality.

Charles Wenz

● *Monograph on the Pharmacology and Toxicology of Nicotine* by A.J. Cohen & F.J.C. Roe is published by the Tobacco Advisory Council, London, at £5.00.

Bazan explains escape from Argentina

Dr Nicolas Bazan, a leading expert on epilepsy, has now made known the circumstances surrounding his dismissal last March from his posts as director of the Argentinian Institute of Biochemical Research at Bahia Blanca and of Professor of Biological Biochemistry at the University of the South (see *Nature* 14 May, p.100). Professor Bazan explained his situation to fellow neurochemists while visiting Britain for the International Neurochemistry Conference in Nottingham, which included a special session on the state of academic freedom and human rights in South America.

Dr Bazan was informed of his dismissal last March. The letters of dismissal were dated 30 December — the day before emergency legislation empowering the government to make such dismissals was due to expire. Dr Bazan, however, suggested that the dismissal notices had been back-dated, since, in the intervening period, he had been allowed to travel to the United States for a meeting of the American Society of Neurochemists, and foreign travel is not normally permitted to Argentinian academics in bad standing with the government.

Dr Bazan stressed that he had never taken part in any political activities, although some years ago he had written letters on behalf of a professor dismissed improperly. He said that true academic life is now impossible in Argentina, and any independence of thought among academics is seen as a dangerous symptom.

In a country where dismissals, detentions without trial and "disappearances" are a regular feature of political life, Dr Bazan was an exception in that his case evoked protests not only from fellow academics at home and abroad but also from several leading Argentinian newspapers, in particular the daily *El Clarin*. The Minister of Education, who had signed the dismissal order, declined to reply to these protests, except by hints that the decision was motivated by considerations of "national security".

Although the Argentinian Academy of Sciences held a meeting of support for Dr Bazan in early June, the build-up of pressure and intimidation against him increased, and at the end of June, he and his family had to flee the country.

Vera Rich

Religion and science

A group of British scientists (who are also Christians) is trying to help the Methodist Church educate its congregation on some moral issues of science and technology policy. Last week the group published a controversial document to guide the church in its task.

The document, called *Shaping Tomorrow*, is essentially a statement of how scientists working in several different fields reconcile their work with their faith. Topics it tackles include nuclear energy policy, the electronics revolution and its impact on employment and the implications of advances in the life sciences for the ethical issues surrounding human reproduction. The idea, according to Mr Edgar Boyes, vice-principal of Luton Industrial College and editor of the document, is partly to provide lay people with facts and partly to put on record the moral judgements of scientists who are enthusiastic about technology and are also Christians.

The idea for the document originally came from scientists working at the United Kingdom Atomic Energy Authority who felt that a grass-roots response was needed to counter certain anti-technology statements made by the clergy. Scientists who are also Christians, they felt, could help the church out of its confusion by providing facts on technological issues and by explaining why they felt justified in pursuing both their careers and their faiths with clear consciences.

Although the document is a Methodist Church publication, the clergy have not greeted it with unanimous approval. In particular, the section on nuclear power is proving contentious. The scientists admit that they have not taken into account judgements contrary to their own but believe that bias may be the key to the document's success in stimulating serious discussion. Debates amongst small groups are already planned in Luton Industrial College and, the scientists hope, churches throughout the country will soon follow suit. **Judy Redfearn**

Third World support

US policy emerges

Washington

The US State Department seems to have given its tentative blessing to a new initiative being promoted by seventeen developing and so-called "middle-tier" countries to support the growth of science and technology in the Third World.

Their goal is to find a way of matching three components: the scientific, technical and institutional needs of the world's poorest nations; the knowledge and know-how that has already been built up by industrialized countries; and access to public and private capital in both these and the oil-producing nations.

But bending to economic and political realities, the hope is that this can be done without provoking the conflicts and confrontations which accompanied recent efforts to carry out the same task in the name of the new International Economic Order — and which have subsequently fallen victim to the stalemate in North-South negotiations illustrated by last month's United Nations Conference on New and Renewable Sources of Energy in Nairobi.

Earlier this year a delegation of ministers and civil servants responsible for national science, technology and economic development policies from ten of the nations visited several oil-producing countries — including Saudi Arabia, Kuwait, the United Arab Emirates, Nigeria and Venezuela — to enlist their support for this new approach (*Nature* May 14, p.103).

Following the apparent success of this mission, an expanded delegation headed by Minister Ben Dhia of Tunisia visited Washington last week for what were later described by its members as "very productive" talks with State Department and other Administration officials.

Well aware that requests for increased foreign aid are unlikely to generate any sympathy from the Reagan Administration's budgeteers — or, indeed, from Congress — the delegation is said to have played down this part of their proposal. However, the State Department seems to have been more interested in those aspects of the new initiative which coincide with its own view that technical assistance to developing nations has an important contribution to make towards national security through reducing political and economic instability in these countries.

In line with this approach, President Reagan's new science adviser, Dr George A. Keyworth, visited Mexico two weeks ago to discuss increased collaboration with the United States in a range of scientific and technological areas, including agriculture, energy, remote sensing and basic sciences.

Dr Keyworth's visit was aimed primarily at reviewing the status of projects initiated under an agreement set up by President Carter. However, a communiqué issued after a meeting with Dr Edmundo Flores, head of the National Council for Science and Technology (CONACYT), emphasized that the goal of the meeting had been to agree on "mechanisms and procedures for the period 1981 to 1983 that adapt the scientific cooperation to the general objectives of bilateral cooperation recently defined by President Ronald Reagan and [Mexican] President José López Portillo."

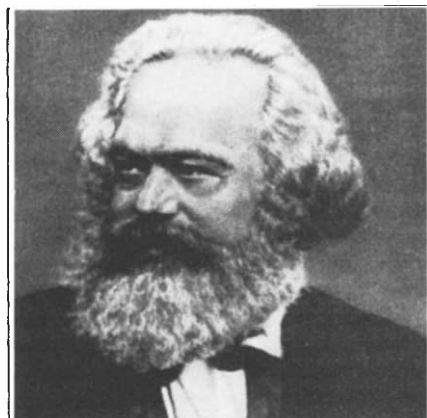
Following their visit to Mexico, the ministerial delegation flew to Bonn for discussion with German government officials, and subsequently to Paris and Brussels for talks with the European Economic Community. The immediate goal is to produce a common position

among the developing countries themselves on their principal needs in building up their scientific and technical capabilities in a form that can be agreed on at a general ministerial meeting being hosted by the Venezuelan government in Caracas early next month.

After that, the next formal step would be to present the proposals to the Second Committee of the United Nations General Assembly, which meets in New York in November. This committee must decide what to do about the Interim Fund for Science and Technology, set up on a two-year basis under the auspices of the United Nations Development Programme (UNDP) at the Vienna Conference, whose mandate will technically run out at the end of December.

Before the committee meets, however, it is widely expected that any proposals agreed to in principle in Caracas will be made known to the heads of state attending the summit meeting on North-South issues taking place at the end of October in Cancun, Mexico, at the invitation of President López Portillo — and with the expected attendance of President Reagan. If this meeting turns out to be successful, those who have been developing the new science and technology initiative — including officials from UNDP — hope that it may prove to be the right size and shape to be endorsed by both developed and developing nations.

David Dickson



Karl Marx — PhD

The Friedrich Schiller University of Jena (GDR) recently discovered new documentary evidence about the awarding of a Doctorate in Philosophy to Karl Marx. A university archivist stumbled on an entry relating to Marx's degree in the records of the Philosophy Faculty for 1841 which has apparently been overlooked by all the biographers and collators of Marx, including the compilers of the *Complete Works of Marx and Engels*. A facsimile of the entry will be reproduced in a history being published to coincide with the university's 425th anniversary in 1983.

Vera Rich

CORRESPONDENCE

Patent share-out

SIR — There is an important omission in your reports (*Nature* 13 August, p.573) on the patents applied for by Stanford and the University of California at San Francisco, based on the work of Cohen and Boyer. The provisions in the patent are an advance over the previous manifestations of the academic-bioindustrial complex, but there is no consideration of the nature of scientific research.

Scientific research is the outstanding example of an endeavour in which the end result by one group of scientists at any one time is the culmination of previous research and papers by many others.

What then is patentable? Is it (1) the result which lends itself to commercial exploitation, or is it (2) all the previous results along that line of inquiry? Under patent law, the two universities can obtain a patent under (1). I maintain that the basic ethos of the scientific community requires that the patent should include (2) — all the previous work and the scientists who did the work. So why should the two universities concerned be sole beneficiaries of the patent when, without the other research going on in that same line of inquiry at other institutions, Cohen and Boyer would never have been able to do their work?

The monetary gains for the patent (if any) should not benefit only the two universities but the whole scientific community. What I therefore suggest is that a universities research fund be set up, by all the institutions at which broadly-based biological research is going on. The administrators of the fund could set the ground rules for membership and for the allocation of monies gained through members' research activities between member institutions.

Only in this way can we preserve that peculiar institution, science, in which the whole is greater than the sum of its parts; in which all labour, or should labour, for the glory of the whole; in which the resultant scientific knowledge, not money, nor personal glory, is the ultimate goal, an institution which has become the hallmark of Western culture and which reflects the yet unknown capabilities of the mind of man.

PHILIP SIEKEVITZ

Rockefeller University, New York, USA

Anti-anti-“racism”?

SIR — I certainly do not want to ascribe to Professor Rose, or anyone else, views that they do not hold, and would like to apologize to him if I have done so. But there is now, I think, a serious possibility of clearing the air. As Professor Rose (*Nature* 23 July, p.286) apparently does not subscribe to the opinion that, within the context of the sociobiology debate, there are no significant genetic differences from place to place within our species (and by implication, that there are no behavioural genetic differences between individuals), then perhaps he would be willing to say that there might be such differences. Since nobody in their right mind would call Professor Rose a racist, a statement of this kind from him would have the beneficial effect of lowering the temperature in the scientific debating chamber.

J.R.G. TURNER

Department of Genetics,
University of Leeds, UK

Consider, please

SIR — It is a noticeable and frequent occurrence at conferences that many people arrive late, enter or leave in the middle of presentations, or depart early, frequently with considerable insensitivity to the speakers and others attending the sessions. The practice is prevalent at conferences with simultaneous (often asynchronous) sessions and is possibly on the increase, though observations at the British Association meeting in York recently preclude the conclusion that the young are responsible; the lead was disquietingly apparent from older generations at the BA meeting, and gives cause to doubt some of the hopes for the future of the BA that you expressed in your editorial (*Nature* 3 September, p.1.)

Is it not time for individuals and conference organizers to work towards some behavioural conventions bearing in mind, though perhaps falling short of, that which prevents someone who is as little as five minutes late for *Don Giovanni* from seeing the entire first act?

MARTIN DAVIES

University of York, UK

Professor Neurath

SIR — Your article “German cancer research: Politics ousts science” (*Nature* 20 August 1981, p.665) is beset with so many inaccuracies and misrepresents the situation in such a deplorable manner that I must ask you to publish the following rebuttal:

(1) It is incorrect to state that I was previously Professor of Biochemistry at the University of Washington and that I have “only been scientific advisor to the University of Seattle”. I was in fact founding chairman of the Department of Biochemistry of the University of Washington, from 1950 until my administrative retirement in 1975, and thereafter I have been Professor of Biochemistry and Associate Director for Scientific Affairs of the Fred Hutchinson Cancer Research Center in Seattle, one of the recognized 21 comprehensive cancer centers in the United States. Your cursory and pejorative description of my professional career reveals a bias which characterizes much of the remainder of your report.

(2) It is incorrect to say that the other directors of the institutes of the German Cancer Research Center were not consulted about my appointment. Rather, my appointment was approved by the Wissenschaftlicher Rat (the internal scientific advisory board) to whom all 8 institute directors belong as voting members, and the Kuratorium (board of trustees), following the statutory process of appointment.

(3) It is not true that the institute pays for my villa. In fact I am paying rent for use of a house which the German Cancer Research Center, with financial aid from a private philanthropic foundation, has rented for that purpose. Finally, although the annual budget of the German Cancer Research Center has been correctly quoted as approximately DM79 million, this sum cannot, even under the most extreme imaginable exchange rates, be equated with \$1,817 million, as stated in your report.

Your entire article represents such shoddy reporting that these inaccuracies alone, including the misquoted name of the Ministry

Director Dr Wolfgang Finke, should convince even the least informed reader of *Nature* of the bias and lack of credibility of this report.

H. NEURATH

German Cancer Research Center,
Heidelberg, FRG

SIR — Your note on German cancer research “Politics ousts science” (*Nature* 20 August, p.665) contains some erroneous statements which I should like to correct:

(1) The German Cancer Research Center in Heidelberg is not “centrally administered by the Bundesministerium für Forschung und Technologie (BMFT)”, but is an independent public foundation according to the laws of the federal state of Baden-Württemberg. It has its own board of directors; a Kuratorium serves as supervisory body; scientific advice is given by an external committee with 12 members and an internal committee with 16 members. However, 90 per cent of the financial contributions to cover the costs of the centre's regular investments and operations are provided for by the BMFT, the remaining 10 per cent come from the federal state of Baden-Württemberg. The federal and state governments together have a veto power only in financial but not in scientific matters.

(2) Professor Neurath was unanimously elected as chairman and scientific member of the board of directors by the Kuratorium in December 1979 after he had been placed at the top of a list of three candidates by the internal scientific advisory committee with 10 of its 16 votes (which include all the directors of the eight institutes of the centre).

(3) Professor Neurath, before coming to Heidelberg, had not only been professor of biochemistry at the University of Washington in Seattle but also director for research (not “only advisor”) at the Fred Hutchinson Cancer Research Center there. He had never “demanded that the institute pay for a villa” and his annual salary was not DM 167,000 — but considerably less.

(4) Professor Neurath did not reject the multidisciplinary, clinically oriented approach of the centre and he did not, with my alleged support (my name being misspelled in your article), propose “to cut back on activities such as nuclear medicine and chemotherapy”. What he proposed was in the long run to concentrate more on research both fundamental and clinically oriented and to reduce the centre's activities in routine work (there were no less than 17,000 cases of patient treatment, mostly diagnosis, in one year in the institute of nuclear medicine alone) by gradually shifting them to other institutions, especially to clinics of the region.

(5) In view of the present situation at the centre the Bundesminister für Forschung und Technologie Dr von Bülow and the Minister für Wissenschaft und Kunst (Land Baden-Württemberg) Professor Engler asked a number of internationally renowned scientists for advice on the future of the centre in order not to oust science there or replace it more and more by routine work but to bring it to full fruition.

WOLFGANG FINKE

Kuratorium des DKFZ,
BMFT, Bonn, FRG

WE accept that our report of Professor Neurath's resignation from the German Cancer Research Center was inaccurate in the respects he lists, and apologize to him and his colleagues for the distress they may have caused — Editor, *Nature*.

Test-tube babies, 1981

R.G. Edwards*

THIS year should prove a turning point for the birth of children by the fertilization of human eggs *in vitro*. Between 15 and 20 such babies will be born in approximately equal numbers in the United Kingdom and Australia, and there will be one or two elsewhere. These methods will be introduced in many countries, primarily to alleviate human infertility. Fundamental aspects of human conception will be analysed and increasing debate will be presumably be given to genetic engineering. This is, therefore, an appropriate time to assess the relevant clinical and scientific issues raised by this work¹.

The first essential: timing of ovulation

I will first discuss the methods involved in timing ovulation for the collection of preovulatory oocytes.

Harvesting preovulatory oocytes is the first of several steps essential for obtaining human embryos. They must be collected during their final stages of maturation just before ovulation occurs, when meiosis is advanced and cortical granules have established the defence against polyspermy. Follicular growth and ovulation must be regulated by endocrine therapy, or natural ovulation must be closely predicted during the menstrual cycle.

The regulation of ovulation is undoubtedly easier. Several follicles can be primed using human menopausal gonadotropin (HMG) or clomiphene early in the menstrual cycle. An endogenous surge of luteinizing hormone (LH) will then induce ovulation. Alternatively, a single injection of human chorionic gonadotropin (HCG, 5,000 IU) can be given between days 11 and 14, according to the follicular response of each patient. Levels of urinary oestrogens of 80–100 μg per day (refs 2,3), or follicular diameters of 1.5–2 cm measured by ultrasound^{4,6} are believed to be appropriate indications to inject HCG. Ovulation can be induced at any desired time of day or night, a considerable help in organizing laboratory or surgical teams for oocyte recovery. Two or more pre-ovulatory oocytes can be collected from many patients, another advantage of stimulating the ovary.

There may be problems to offset these advantages. Wide variations exist in the rate of growth of individual follicles in each

patient, revealed by the different levels of steroids in follicular fluids⁷ and by variations in embryonic growth when super-ovulation techniques are applied to animals. Some patients fail to respond to clomiphene. Others produce increasingly large amounts of oestrogens as several follicles grow, and their endogenous LH

stimulation can also distort the menstrual cycle, inducing a short luteal phase and a disorganized endometrium, both incompatible with establishing pregnancy¹². An average of eight cycles of treatment with clomiphene is needed for oligomenorrhoeic women to conceive naturally³. Such disadvantages may be greater with

Between fifteen and twenty babies will be born this year after the *in vitro* fertilization of human eggs. Many of the essential steps now have high rates of success, including the recovery of preovulatory oocytes, and fertilization and embryo cleavage *in vitro*. Implantation of the embryo following its replacement in the mother remains the major difficulty. Some implications of the work are discussed.

surge stimulates ovulation. A difficult situation occurs in patients with moderate or high levels of oestrogens and no endogenous surge of LH (Fig. 1). As in other tissues^{8–11}, clomiphene may have depleted cytoplasmic oestrogen receptors in the pituitary gland of such patients over several days, so preventing the LH surge in response to rising levels of oestrogens. HCG must be given at some arbitrary time, before follicles become atretic, yet while it is uncertain if the patient will have her own endogenous LH surge. Ovarian

HMG than with clomiphene.

The alternative is to monitor the approach of ovulation during the natural menstrual cycle, and then aspirate the single preovulatory oocyte. This method was used for the conception of the first child by fertilization *in vitro*¹², and is widely used in our practice today. Plasma or urinary oestrogens are used to assess follicle growth, and the onset of the LH surge at mid-cycle provides a warning of the approach of ovulation.

The disadvantages are obvious. There is

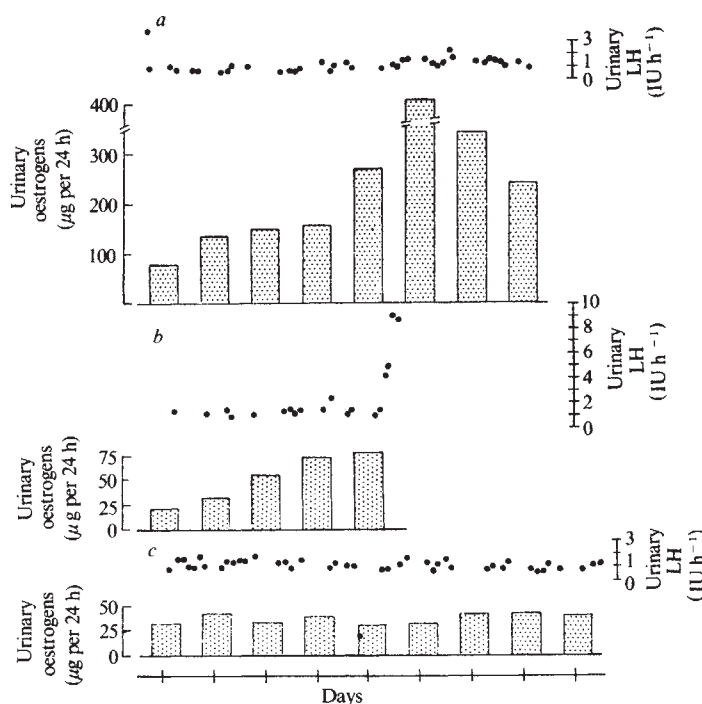


Fig. 1 Response of patients to clomiphene. *a*, Levels of urinary oestrogens rose considerably, and then fell in the absence of an endogenous LH surge. *b*, Rising levels of urinary oestrogens followed by surge of LH in urine. *c*, No response in urinary oestrogens and no surge. □, Urinary oestrogens; •, urinary LH, measured by the Hi-Govanis kit.

*Physiological Laboratory, Cambridge University, Cambridge CB2 3EG, UK, and Bourn Hall, Cambridge CB3 7TR, UK.

Table 1 Aspiration of oocytes from preovulatory follicles

	Natural cycle*	Natural and clomiphene cycle ²⁰	Clomiphene cycle ⁶
No. of patients	122	—	—
No. with accessible ovaries	109	—	—
No. of preovulatory follicles aspirated	109	172	107
No. (and %) of oocytes collected	95 (88%)	110 (64%)	96 (89%)

*Recent series of 122 patients in Bourn Hall, Cambridge. Their average age was 33.9 yr.

usually only one preovulatory oocyte, and any pathological conditions in the ovary or abdomen will limit the chances of collecting it. The LH surge is less predictable than an injection of HCG, hence more difficulty arises in organizing laboratory and clinical staff to collect the oocyte. Some difficulties have proved less than feared. Repeated blood sampling may provide a reliable guide for assaying LH^{6,13-15}, but is unnecessary, because urine

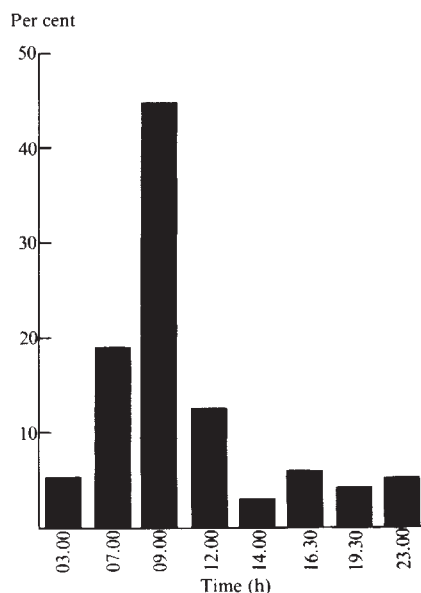


Fig. 2 Diurnal rhythm in the onset of the urinary LH surge in women. The time shows the initial increase in levels of LH which was followed by a sustained rise (see Figs 1b and 3).

samples are suitable for rapid assays of LH using the kit Hi-Gonavis (Mochida Pharmaceuticals)¹² and for oestrogens. Fortunately, the LH surge begins in early morning in almost three-quarters of the women (Fig. 2), which is most convenient for the collection of preovulatory oocytes 24–28 h later. This 'critical period' in the LH surge in women resembles the situation in rats^{16,17}, and its distinct diurnal component must modify the feedback effects of ovarian oestrogens and LH releasing hormone on the pituitary gland¹⁸. If women discharge their LH at other times of day, their oocytes must sometimes be aspirated less than 24 h later and cultured *in vitro* to complete maturation.

Fertility may be higher during the natural cycle than after ovarian stimu-

lation, an obvious advantage in establishing pregnancy. Several surveys have revealed a 1 in 4 chance of pregnancy during unprotected intercourse in any menstrual cycle, but even this low rate may be higher than during induced cycles. Clomiphene may be indicated in patients with irregular or prolonged cycles.

Aspiration of oocytes

A double aspirating needle or two separate needles are used, one channel being used for aspiration and the other to flush out the follicle if the oocyte is not collected^{19,20}. The flushing solution may contain heparin, to prevent clotting within the follicle.

The highest rates of collection are achieved 32 h after the injection of HCG, or 26 h after the rise of LH in urine (Table 1). It is essential to time the onset of ovulation correctly. Should HCG be given after an endogenous surge of LH, ovulation may occur before aspiration begins. Diurnal rhythms in tonic LH release, sometimes reaching low surge levels over a few hours, can confuse the correct timing of the LH surge (Fig. 3). Pelvic adhesions, hydrosalpinx, cystic follicles, endometriosis and other ovarian conditions impair the collection of oocytes, and preliminary laparoscopy may be required to alleviate these conditions. If the ovary is accessible, oocytes can be collected during the natural cycle from almost 90% of patients, laparoscopy being completed within a few minutes (Table 1). None of our patients had ovulated when laparoscopy was performed, and each of them had a large preovulatory follicle.

Preovulatory oocytes can be identified quickly. They are embedded in a viscous follicular fluid, to a diameter of 0.5 cm or more and can be seen by eye². The viscosity of the cumulus mass serves as a guide to the stage of maturity of the oocyte. A few hours in culture in the presence of some

follicular fluid helps to complete maturation, especially in those deliberately collected less than 26 h after the LH rise, which appear 'unripe'.

Fertilization and cleavage *in vitro*

Provided there are no pathological conditions in the husband's spermatozoa, almost 90% of the preovulatory oocytes aspirated during the natural cycle can be fertilized *in vitro* (Table 2). The conditions of culture are rapidly becoming standardized^{3,6,7,12}. Simple media are used, including Earle's solution with pyruvate, a medium designed to support mouse embryos *in vitro*^{21,22}, and Ham's F10, each supplemented with serum albumin or homologous human serum (7.5–8.6%). We prefer Earle's solution containing pyruvate, with serum from each patient (Table 3)¹². Sperm numbers vary between 10⁵ and 10⁶ per ml, or between 1 and 5 × 10⁵ living spermatozoa per ml. Droplets of medium held under paraffin oil or small culture tubes can be used for fertilization. Our present practice is to begin fertilization in droplets of medium under oil, adding 1.5–2 × 10⁵ living spermatozoa per ml between 3 and 4 h after aspiration of oocytes; the oocytes are placed in tubes some hours later when most of their cumulus cells have been shed¹².

The oocytes are fertilized within a few hours. Some have been fertilized following a re-insemination 48 h later and grew normally to the eight-cell stage (J.M. Purdy and R.G.E., unpublished). Conditions such as spermagglutination, viscous seminal plasma and erratic sperm movement reduce the chances of fertilization (Table 2). Almost all eggs are monospermic, indicating that the block to polyspermy is efficient^{3,6,23}.

Most embryos cleave normally. They tolerate a wide variety of culture media, similar to those used for fertilization. Serum is almost universally added; in our work, we use 15% v/v of homologous serum¹². Cleavage times are remarkably similar despite the varying concentration of energy sources in different media (Table 3). There have been occasional reports of cell fragments in cleaving eggs, although similar conditions have been found in embryos flushed from the reproductive tract and may not be pathological^{24,25}. Many embryos grow to morulae and

Table 2 Fertilization of human eggs *in vitro*

	Patients with occluded oviducts		Patients with idiopathic infertility ⁶
	Bourn Hall*	Melbourne ⁶	
Total no. patients	95	—	—
No. with pathological conditions:			
Spermagglutination cells or debris in semen	5	—	—
Abnormal movement of spermatozoa	3	—	—
Viscous seminal plasma	1	—	—
Remainder	86	40	—
No. (and %) fertilized	76 (88%)	37 (92%)	35%

*Recent survey of 122 patients (see Table 1).

blastocysts, and escape from the zona pellucida *in vitro*^{3,6}, although some apparently develop abnormally during these later stages of growth⁶. The fastest-growing embryos are apparently the most successful in establishing pregnancy^{6,26}. Cine films of cleavage with IR photography may enable embryos to be examined for their nuclear structure before they are replanted in the mother²⁷.

Replanting embryos

Implantation is the most difficult and unpredictable stage. In animals, rates of implantation are high when embryos are replaced surgically (> 70%), but lower if replaced non-surgically via the cervix (~50%)^{28,29}. Almost all human embryos have been replaced transcervically, and approximately one-quarter of them have

Table 3 Composition of the media used for fertilization and cleavage of human eggs *in vitro* (g l⁻¹)

	Modified Earles ¹²	Media for mouse eggs ^{21,22}	Ham's F10*
CaCl ₂ ·2H ₂ O	0.2649	—	0.441
KCl	0.400	0.356	0.285
MgSO ₄ ·7H ₂ O	0.200	0.294	0.1527
NaCl	6.800	5.140	7.400
NaH ₂ PO ₄ ·2H ₂ O	0.1583	—	—
Na ₂ HPO ₄	—	—	0.156
KH ₂ PO ₄	—	0.162	—
NaHCO ₃	2.1	1.9	1.2
Ca lactate	—	0.527	—
Na pyruvate	0.011	0.025	0.110
Glucose	1.000	1.000	1.100
Na lactate (60% syrup)	—	3.7 ml l ⁻¹	—

Embryos growing most rapidly are two-cell by 21 h after insemination, four-cell at 40 h, and eight-cell at 44–54 h (refs 3, 23).

*Also contains CuSO₄, FeSO₄, ZnSO₄, amino acids, etc.

implanted in some series (Table 4). Embryos might be best replaced during the evening¹², because the spontaneous contractility of the human uterus is lower at night^{30,31}. Disorders in the luteal phase, including progesterone deficiency and a short luteal phase, could preclude implantation (Table 4). Some implanted embryos live for only a short time after the expected return of menstruation, and are identified by a transitory rise in HCGβ (ref. 3) (Table 4).

Why do so few embryos implant? Perhaps 20% or thereabouts is all that can be expected, since it is similar to the incidence of natural human fertility reported in seral surveys. This view seems to be pessimistic. Some embryos may be lost or infected if there are difficulties in passing a catheter through the internal os. An outer metal cannula can be useful in such cases, but pregnancy rates are lower (Table 4). A Teflon catheter with a smooth tip and side aperture evidently passes easily through the internal os⁶, but may require the use of a tenaculum on the cervix. Implantations have occurred after replacing all stages between the two-cell and blastocyst, but there are insufficient data as yet on the optimal stage for replacement²⁶.

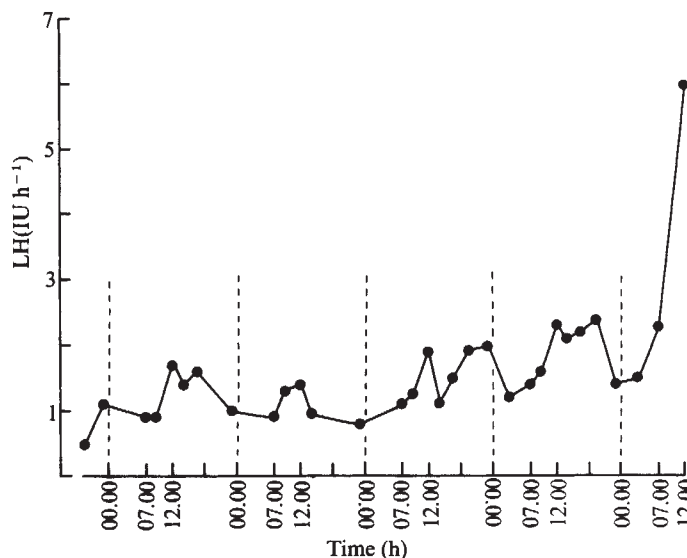


Fig. 3 Diurnal variations in the levels of urinary LH in women. 'Tonic' levels rise each morning, then decline. The LH surge began during the morning in this patient.

Physiological disorders might prevent implantation. Vaginal distension during replacement could invoke discharges of prolactin, and a catheter passed through the uterine cavity may invoke a premature decidualization of the endometrium. Slight myometrial contractions could expel embryos soon after their replacement and might be inhibited using β_2 -mimetics^{30,31}. Restricting the volume of medium might encourage implantation (0.03–0.10 ml are used at present). Surgical transfer might circumvent these problems, but a second anaesthesia will be needed soon after that used for oocyte recovery, and the myometrium and endometrium will undoubtedly bleed when a needle is passed through them. In rhesus monkeys, 11 out of 15 embryos replaced surgically in the oviduct, and 2 of 8 replaced in the uterus developed to advanced stages of gestation³².

Growth of human fetuses after replacement in the mother

At least 12 children have been born after fertilization *in vitro*, 3 in the initial series in

Oldham³³, 7 in Australia^{6,34}, and 2 recently in the United Kingdom. Two of these were born prematurely at approximately 20 weeks, soon after amniocentesis. The children have been healthy, although a male twin required corrective surgery, and several current pregnancies appear to be progressing normally^{6,26}. Details of all pregnancies are being recorded in an international register to evaluate any risks to the fetuses and the causes of abortion or fetal death.

Between one-quarter and two-fifths of the fetuses arising from fertilization *in vitro* die *in utero*, mostly in the first trimester. Maternal age (late thirties and early forties) may be a disposing factor in some cases. One dead fetus was triploid³³, the others have not been karyotyped. Triploidy may not be serious quantitatively, as the vast majority of fertilized eggs are monospermic. Most triploid fetuses arising during fertilization *in vivo* are caused by dispermy, fewer being due to fertilization by a diploid spermatozoon or cleavage errors in the embryo³⁵. Nor

Table 4 Pregnancies after replacing human embryos transcervically (same group of patients as in Tables 1 and 2)

	Single catheter 51	Double catheter 23
No. of patients		
No. with deficient luteal phases:		
Short luteal phase*	2*	0
Progesterone deficiency †	1	3
No. with delayed return to menstruation‡:		
Brief elevated levels of HCGβ	2	0
Brief elevated levels of HCG/LH	3	3
No. pregnant 4 weeks after replacement	12 (23.1%)	2 (8.7%)

* Luteal phase of 12 days or less; these patients had luteal phases of 5 and 12 days, respectively, one also having progesterone deficiency.

† These patients had between 0.4 and 2.7 ng ml⁻¹ on days 7 or 8 of the luteal phase.

‡ Some other patients showed a delayed return to menstruation but no evidence was found of elevated HCGβ or HCG/LH.

Table 5 Results on a recent series of 122 patients with tubal occlusion

No. of patients	122
Failure to collect oocyte:	
Method failure	14
Adhesions, endometriosis, etc.	13
(Table 1)	27
No. with preovulatory oocytes	95
Failure of fertilization	
Method failure	9 + 1?
Pathological spermatozoa	9
(Table 2)	19
Embryos not replaced	2
No. of embryos replaced	74
Failure of implantation:	
Short luteal phase/ progesterone deficiency	6
Method failure*	49
	55
Indications of pregnancy:	
Delayed RTM: early abortion?	5
Pregnant 4 weeks after replacement	14

The natural menstrual cycle was monitored. RTM, return to menstruation.

*See Table 4.

should trisomy be more frequent after fertilization *in vitro*. Most human trisomies arise during the first meiotic division of the oocyte^{36,37}, a stage which is virtually complete before the oocytes are aspirated. Others arise during the second meiotic division, that is, after fertilization, or during cleavage of the embryos, but there is no record of their incidence *in vitro*. Amniocentesis should be performed on all fetuses arising through fertilization *in vitro*, if the parents agree, until the risks of trisomy have been assessed.

Some fetuses may die *in utero* through physiological or embryological factors associated with fertilization *in vitro*. These could include damage to the uterine wall during replacement of the embryos, the introduction of cervical flora into the uterine cavity and the implantation of the embryo low in the uterine canal³. Short or deficient luteal phases arising after ovarian stimulation, or through the withdrawal of too many granulosa cells from the preovulatory follicle occur in very few patients (see Table 4)³⁸. Abnormal embryonic differentiation *in vitro* might have led to the transitory secretion of HCG in some patients, as the fetus developed into a 'blighted ovum' or trophoblastic vesicles, resulting in its early death. Such conditions arise following conception *in vivo*^{39,40}.

Genetic engineering

Arguments sometimes raised against the introduction of fertilization *in vitro* into clinical practice involve the possibility of genetic engineering — chimaeras, hybrids and cloning embryos. These issues are quite distinct from the alleviation of infertility. Three cloned mice have been born, using nuclei from blastocysts⁴¹, and others after the use of nuclei from the embryonic ectoderm and proximal endoderm of 7-day mouse embryos, showing that the developmental potential of nuclei from earlier embryos is retained in these cell types⁴².

The thought of cloned human embryos identical to a pre-existing individual is not attractive. Yet, in a sense, uniparental human embryos largely similar to the father already exist. Hydatidiform moles are the remains of diploid androgenetic fetuses, in which the trophoblast proliferates over several weeks to form large grape-like vesicles. Such fetuses evidently arise due to the expulsion of the female pronucleus from the fertilized egg⁴³⁻⁴⁶. The sperm chromosomes are doubled and the embryo expresses paternal chromosomes only. Rare hydatidiform moles may even retain much of the father's heterozygosity, since they could arise from a diploid spermatozoon. The reason androgenetic embryos undergo hydatidiform changes is not understood. Perhaps the embryonic cells die early, or the mother 'rejects' a fetus lacking her own antigens⁴⁶; nevertheless, androgenetic mouse embryos develop normally to full term⁴⁷. Human gynogenones could also arise through similar processes involving expulsion of the male pronucleus from the egg, although no searches have yet been made for them before or after birth. Uniparental embryos could arise through delayed syngamy and an enlarged pronucleus, seen in two human eggs fertilized *in vitro*.

Subtle forms of genetic engineering have been introduced. DNA fragments containing the gene for human insulin were injected into pronucleate mouse eggs, and the gene was identified in tissues of fetuses at the 18th day of pregnancy⁴². The DNA evidently replicated and was inserted into

the genome of the fetus, but there is no information as to whether it was transcribed. There is apparently no knowledge of the potential value of such treatments in preventing the expression of inherited conditions such as diabetes.

Prospects

Many children will soon be born after the fertilization of human eggs *in vitro*. We have established more than 40 pregnancies since resuming work during the past 9 months, and the majority are surviving. This is most encouraging for those couples who could not be offered any other form of corrective surgery and have so far been without effective treatment (Table 5). The method can be carried out several times on the same patient, and success rates should soon exceed some forms of oviductal surgery. If ovarian stimulation is used, 'spare embryos' may be available for embryological studies and one embryo has grown for 9 days *in vitro* until stage 5a (ref. 3). The frozen storage of human embryos still appears to be distant. Some of the fathers were oligospermic, and patients with idiopathic (unexplained) infertility, hostile cervical mucus, incompetent cervix, antibodies against the zona pellucida, might also be helped. Complex disorders leading to the abnormal growth of pronuclei can be investigated⁴⁸.

I thank Jean Purdy and Patrick Steptoe for their help at all stages of this work, and Simon Fishel for his comments on the manuscript.

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NEWS AND VIEWS

Birds of paradise and the theory of sexual selection

from Jared M. Diamond

THE most bizarrely beautiful birds are the birds of paradise (family Paradisaeidae), which live on New Guinea and adjacent islands. Adult males bear specialized ornamental feathers that include coloured grass-like plumes, false wings and shields and capes, flag-tipped wires growing out of the crown or tail, long wires growing from the eyebrows with a dozen ornaments like pieces of flat blue plastic and tails several times as long as the body. How could natural selection produce such creatures?

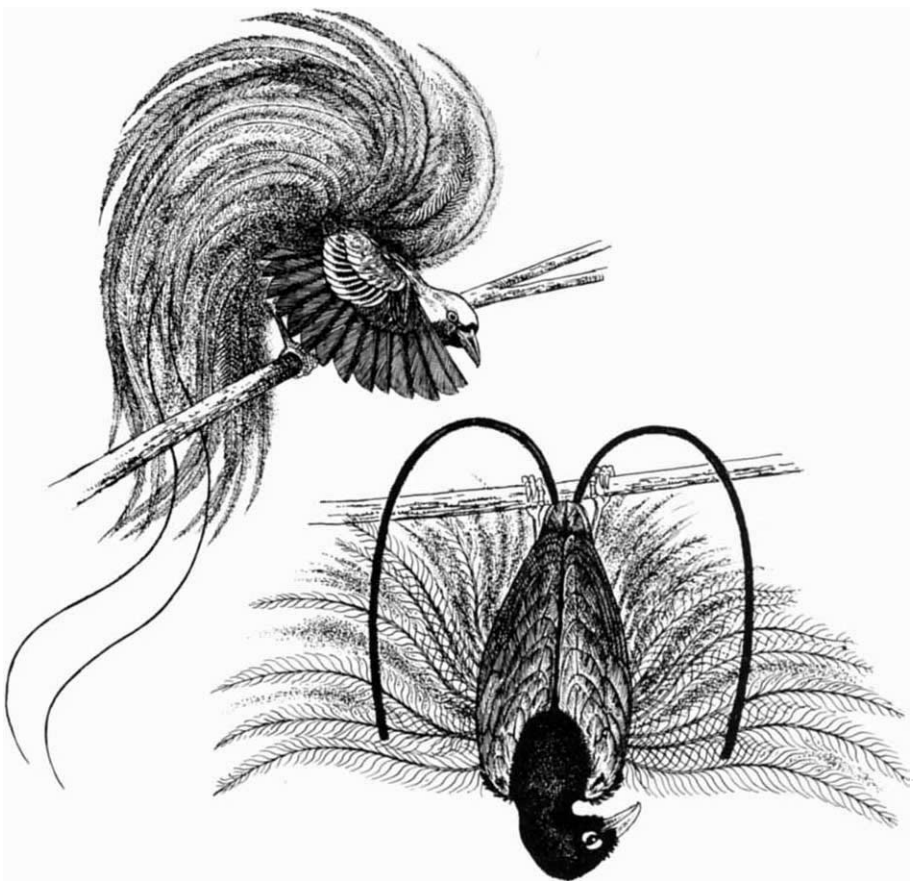
Interest in this question is motivated not only by the beauty of the birds, but also by their relevance to understanding the broader problem of sexual selection. Many species are sexually monomorphic (males and females similar in appearance), but others, including humans (compare with ref. 1) and birds of paradise, are sexually dimorphic. Darwin devoted an entire book² to arguing that some sex-linked characters of sexually dimorphic species arise through intraspecific competition for mates. He termed this process sexual selection, as opposed to natural selection (differential survival of self and offspring).

For centuries after the first bird of paradise skins reached Europe with surviving crew from Magellan's voyage of circumnavigation, western knowledge of these birds was confined to skins obtained through Moluccan middlemen from New Guinea hunters. Most of the 42 bird of paradise species live in the rugged mountainous interior of New Guinea, where physical hardships of ornithological exploration ultimately cost Hunstein, Stalker, Gilliard and others their lives. Until 1824, when a French explorer became the first European to see a bird of paradise in the wild in New Guinea, Europeans believed that the birds spent their entire

lives flying high in the sky, nourishing themselves with the nectar of heaven and descending to Earth only to die (hence the name birds of paradise). Modern exploration showed their actual behaviour to be hardly less strange — adult males of some species live in clans apart from females and hang upside down or

cooperate in duetting pairs to woo females.

Recent advances in our understanding of bird of paradise behaviour⁴⁻⁸ builds on the early work of the late E.T. Gilliard³ of the American Museum of Natural History. Two recent papers by Mary LeCroy of the same museum are of particular importance. In the first of these papers⁹,



Left, a male-to-male display ('charging display') in the Lesser Bird of Paradise, *Paradisaea minor*. Right, a male-to-female display ('inverted display') in the Blue Bird of Paradise, *Paradisaea rudolphi*, by a male hanging upside-down from a branch. Drawings by Juan Barbaris, from LeCroy¹⁰.

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LeCroy collaborated with Alfred Kulupi of the Papua New Guinea Wildlife Division and photographer William Peckover to study Goldie's Bird of Paradise (*Paradisaea decora*) in the mountains of Fergusson Island. In the second¹⁰ she analyses displays and evolution of all seven species of genus *Paradisaea*, the 'ultimate birds of paradise' (the ones with the most extreme social system and advanced speciation).

The paradisaeas are among the relatively few bird species independently to have evolved a peculiar social system based on 'arenas' or 'leks'. Other arena birds include the ruff of Europe (a sandpiper; see ref. 11), grouse¹² of Europe and North America, manakins¹³ and cotingas¹⁴ of the neotropics, and three other bird of paradise genera. Unlike most birds, in which male and female form a pair bond and share the work of nesting, arena birds form no pair bond and females do all the nest construction and feeding of young. Males spend much of the year in clans without females, at traditional arena sites where they display and establish a dominance hierarchy. Females visit the arenas and consort with males only briefly, to copulate. As males do not visit the nest and can therefore afford to be conspicuous without betraying the nest location, only females have cryptic plumage. Adult males rapidly evolve elaborate plumes through sexual selection (see below), accelerated by the fact that only a small percentage of the males in a clan is responsible for most matings. Interspecific hybrids between morphologically very distinct arena species may arise when a receptive female wanders into an arena of the wrong species as reproductive isolating mechanisms do not keep pace with morphological change in males. Sexual dimorphism involves size as well as plumage: adult males are much larger than females. The population consists of many fewer plumed adult males than females or unplumed males, possibly because plume development is hormonally inhibited in subordinate males. Adult males have loud calls to assemble each other at the arena, to attract females and to communicate with clans at neighbouring arenas. Similar social systems have arisen independently in numerous mammalian, frog and insect species.

As an example of arena courtship, consider what LeCroy, Kulupi and Peckover discovered for *Paradisaea decora*. The arena consists of four tall trees within 100 metres, under which grow fruit trees, on which the birds feed. The fruit trees have grown from seeds dropped by the birds while they eat the fruits in the display trees. Each display tree is occupied by a pair of plumed males and is visited by unplumed males plus plumed males from adjacent trees. The pair of males display facing each other while singing a duet and chasing off unplumed males. When a female appears at the tree, the plumed males there launch themselves into a

different set of displays. Other plumed males leave until only the duetting pair remains. At the peak of the display, one duetter steps aside and stands by quietly, while the other continues the display. Unplumed males crowd around the female and copulate with her very briefly as she begins soliciting and quivers her wings. Finally, the plumed male comes to the female and proceeds to a lengthy, ritualized copulation.

To appreciate the significance of these observations, recall what Darwin theorized about sexual selection and what has been debated ever since. He reasoned that sexual selection involves two distinct struggles: one between males to dominate, drive off, or kill their rivals, with females remaining uninvolved and males providing the selective force; the other, between males to attract females, with females providing the selective force (the same two hypotheses can apply with the sexes reversed). Studies in Hawaiian *Drosophila* have proved a role for both forces¹⁵. Behaviour of arena birds has generally been interpreted in terms of selection by females.

LeCroy recognized that interactions among bird of paradise males are far more important than realized by previous observers, who did not discern that males adopt distinct displays towards females and other males and who had difficulty distinguishing between females and unplumed males. Displays among adult male paradisaeas are noisy and full of motion, involving elements that LeCroy terms charging, zigzagging, duetting and wing posing. These displays go on for much of the year and establish a dominance hierarchy among males. Displays of males to females involve silent, static posturing, such as inverted display (hanging upside-down from a branch). Most important, LeCroy, Kulupi and Peckover saw no evidence in *Paradisaea decora* that females made any selection among males. Rather, the males had it all prearranged among themselves: when a female appeared at a group display of several plumed males, the other males one by one stepped back, leaving only the dominant male to mate with the female. The winner had already

been determined by the males' struggles while setting up the dominance hierarchy. Thus, in LeCroy's view, the immediate function of bizarre plumes and large body size in adult male birds of paradise is not so much to woo females as to dominate other males.

Gilliard's studies of numerous bird of paradise species brought them from the realm of exotic aberrations to the forefront of sociobiology. LeCroy's work goes further and permits her to frame a host of important unsolved questions. What are the genetic relationships among the males of a clan, or between duetting 'brothers'? It seems incredible that the plumed *Paradisaea decora* male chases off unplumed males until the peak of the display and then lets them copulate with the female. Is it because their copulations are brief and ineffective, or is it only the last sperm injection that fertilizes (as in chickens)¹⁶, or are the unplumed males the younger brothers of the plumed male? Why do subordinate males join a clan at all — to learn by apprenticeship, or to get matings by stealth? How stable is the male dominance hierarchy, and is recruitment to the top from subdominant plumed males moving up or from unplumed males maturing? Males are polygynous; are females polyandrous? What percentage of copulations goes to the dominant male? (Dinsmore estimated 100 per cent for *Paradisaea apoda*¹⁴). It is also questionable whether inbreeding is selected through females bringing their daughters to the arena where their father is dominant, as Smith¹⁷ proposed for a polygynous mammal, the Fallow Deer. What are the ecological factors that permit or select for arena behaviour, which has arisen independently numerous times (compare with ref. 18); and to what extent do LeCroy's findings for the 'ultimate birds of paradise', the species of *Paradisaea*, apply to other arena-displaying birds of paradise (*Astrapia*, *Parotia*, *Semioptera* and possibly others)? Some birds of paradise, including perhaps the lovely Blue Bird of Paradise *Paradisaea rudolphi*, are monogamous and territorial; is this a primitive or secondarily derived condition? Are birds of paradise embarked on a maladaptive course of 'runaway evolution' heading for extinction¹⁹? Is there any relationship between the Paradisaeidae and the equally remarkable bower birds (Ptilonorhynchidae) of the same geographical region? It will also be interesting to see whether better understanding of evolution in birds of paradise, through behavioural study, will help our understanding of their adaptive radiation in feeding, of which they furnish an as-yet little studied example rivalling that of Darwin's finches²⁰.

Progress towards solving these questions awaits banding studies in the wilds of New Guinea, through which individual birds at arenas can be recognized and their genetic relatedness determined. [I]

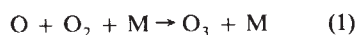
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Solar Mesosphere Explorer to study ozone

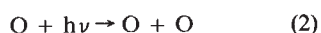
from Charles A. Barth

THE ozone in the Earth's atmosphere continues to provoke a degree of scientific and political controversy which reflects our ignorance of its chemical behaviour. The Solar Mesosphere Explorer Satellite (SME), to be launched on October 4, 1981 should provide a new source of information which will help to clear up many long-standing puzzles*. For the first time, continuous simultaneous stratospheric measurements will become available of the stratospheric content of ozone, of other constituents important to ozone chemistry and of the UV radiation from the Sun. It is hoped that anthropogenic effects on ozone will be detectable as residual changes. The satellite is expected to be in operation for at least one year and perhaps as long as three years.

Ozone is produced in the upper atmosphere from the association of atomic oxygen with molecular oxygen in a three-body reaction:



The atomic oxygen is produced in turn by the photodissociation of molecular oxygen by solar UV radiation of wavelength shorter than 242 nm:



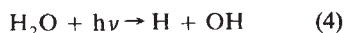
The ozone is also photodissociated by solar UV radiation in the wavelengths between 200 and 310 nm. At this time of moderate solar activity, changes may occur in the solar UV irradiance during a 27-day solar rotation. Instruments on the SME will simultaneously monitor these two regions of the solar spectrum and the distribution of ozone from 30 to 85 km in the upper stratosphere and mesosphere. The altitude distribution of ozone will be measured simultaneously by three limb-scanning instruments: an UV spectrometer measuring the back-scattered radiation between 255 and 310 nm, an IR radiometer

measuring thermal emission at 9.6- μm and an IR spectrometer measuring the 1.27- μm airglow emission arising from radioactive decay of an excited state of O_2 that is the result of ozone photodissociation

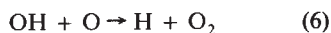
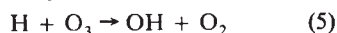


Changes in the solar UV flux and ozone density may cause the temperature of the atmosphere to change. Temperature changes in turn cause ozone changes because the rate of some of the chemical reactions, particularly reaction (1), are temperature dependent. The IR radiometer on the SME will measure temperature and pressure as a function of altitude by measuring the thermal emission from two portions of the 15- μm CO_2 band.

A further complication in tracing the effect of changes in solar radiation on the ozone density occurs in the upper part of the mesosphere. Solar Ly α radiation at 121.6 nm, which may vary significantly during a solar rotation, effectively photodissociates water vapour:



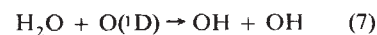
The products of this reaction react with ozone and atomic oxygen at these heights in a catalytic cycle that consumes ozone:



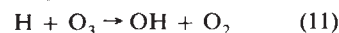
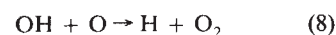
To follow the cause and effect of this relationship, the solar UV spectrometer on the SME will measure solar Ly α radiation and the IR radiometer will measure the water vapour density in the atmosphere by measuring thermal emission in the 6.3- μm band.

In the upper stratosphere, water vapour is dissociated by the reaction with excited

oxygen atoms $\text{O}(^1\text{D})$ which are produced from the photodissociation of ozone [reaction (3)]:

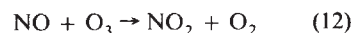


The hydroxyl radicals participate in a catalytic cycle in the upper stratosphere that consumes atomic oxygen and reduces the ozone density

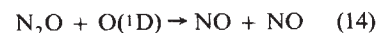


Changes in the amount of water vapour are therefore expected to cause changes in the ozone density in the upper stratosphere and mesosphere. The limb-scanning instruments on the SME will simultaneously measure the density of water and ozone as a function of altitude.

Nitric oxide and nitrogen dioxide form a catalytic cycle in the stratosphere which consumes ozone and atomic oxygen:



Nitric oxide enters the stratosphere through the reaction of nitrous oxide with $\text{O}(^1\text{D})$ atoms that are formed from the photodissociation of ozone:



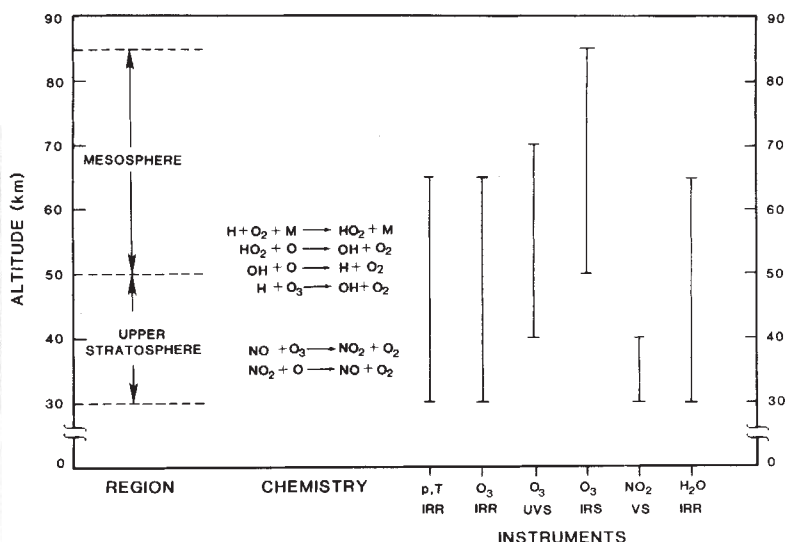
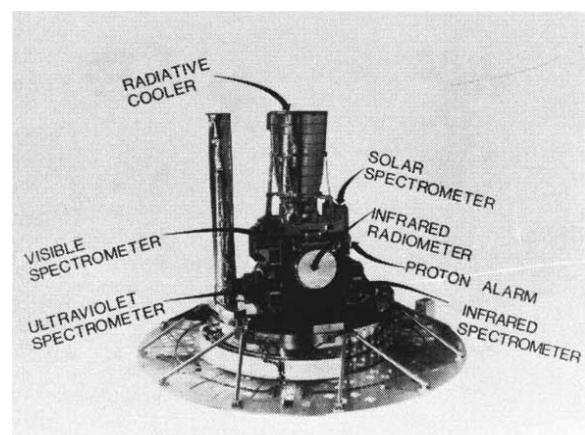
Nitrogen dioxide may be released in the stratosphere from the thermal decomposition of nitrogen pentoxide, an upper atmosphere reservoir of oxides of nitrogen:



A visible-light spectrometer will measure the density of nitrogen dioxide in the stratosphere by measuring the differential absorption of scattered sunlight at two wavelengths near 440 nm.

Energetic particle outbursts on the Sun

The Solar Mesosphere Explorer satellite (below) and a table (right) showing the regions of the atmosphere in which the different reactions occur and the instruments that will be used to monitor them.



lead to the penetration of charged particles into the mesosphere in the polar regions of the Earth (solar proton or PCA events). These charged particles cause the dissociation of water vapour to create atomic hydrogen and hydroxyl radicals which catalytically destroy ozone. At lower levels the charged particles create oxides of nitrogen from the molecular nitrogen and

molecular oxygen of the atmosphere. If a significant number of solar proton events occur during the operational lifetime of the SME, the instruments will be used to determine the relationship between the magnitude of decrease in ozone and the flux and energy of the solar protons and the role of water vapour in the solar proton destruction of ozone. □

*The orbit of SME is planned to be circular at an altitude of 540 km and sun-synchronous with the Equator crossing occurring at 3 p.m. local time. The four limb-scanning instruments will view the atmosphere in the plane of the orbit. The satellite will be operated from a control center at the Laboratory for Atmospheric and Space Physics, University of Colorado, Boulder. The SME investigator team consists of scientists from the Laboratory for Atmospheric and Space Physics and the Department of Astro-Geophysics of the University of Colorado, the National Center for Atmospheric Research, the National Oceanic and Atmospheric Administration Aeronomy Laboratory and the Jet Propulsion Laboratory. The mission is a NASA project, managed by the Jet Propulsion Laboratory.

The significance of unique or rare meteorites

from Robert Hutchison

MOST meteorites have chemical properties which place them in one of 27 groups, each containing five or more members. Much of our knowledge of the chemistry and early history of planetary bodies in the Solar System is based on these common types of meteorite, but it is the rarities which often provide data vital to planetary science. A prime example is the Angra dos Reis (stone) meteorite which fell into 2 m of seawater in Brasil in 1869. Two pieces, together weighing approximately 1.5 kg, were recovered by a diver the day after its fall. The importance of the meteorite is such that a consortium was set up to study it in the mid 1970s¹.

The detailed petrographical and mineralogical work of Prinz and colleagues showed the meteorite to be a cumulate of exceptionally Ca-rich pyroxene crystals. In addition, their analyses confirmed the earlier conclusion of Hutchison² that the mineral assemblage is indicative of a high degree of silica undersaturation. Ma and co-workers calculated from the trace element content of the bulk meteorite and of mineral separates that the meteorite represents a late stage in the fractional crystallization of a liquid which formed as a partial melt from an olivine-orthopyroxene-clinopyroxene source. This, however, poses a problem, for production of an undersaturated liquid from orthopyroxene-bearing starting material requires a pressure > 7 kbar, and hence a lunar-sized parent planet. Various rare gas, isotopic and fission-track studies indicate that Angra dos Reis (stone) crystallized close to 4.55 Gyr ago and cooled faster than 70K Myr⁻¹. This age is within experimental error of that of the eucrites (see, for example, ref. 3), basaltic meteorites which crystallized from tholeiitic liquids⁴. Thus, both saturated and undersaturated silicate liquids were extant at about the same time, an observation consistent with the co-existence of orthopyroxene-bearing and nepheline-normative chondrules in unequilibrated ordinary chondrites (see,

for example, refs 5,6). Angra dos Reis (stone) raises the question of whether lunar-sized objects may be represented in meteorite collections. Other possibilities are that silica-undersaturated planetesimals existed, or a non-magmatic process, such as vapour fractionation (loss) of silica, may have taken place.

At the other extreme from Angra dos Reis (stone), but equally problematical, are Nakhla and two similar meteorites. Nakhla is a meteorite shower which fell in Egypt in 1911 and comprises an unshocked, coarse-grained pyroxene-olivine cumulate (see ref. 7 and refs therein) with a crystallization age of 1.24 Gyr⁸. Samarium-neodymium systematics are consistent with the age of 1.24 Gyr, but they also indicate that the parent material had a three (or more)-stage fractionation history if a chondritic composition is assumed for the original source⁹. The complete resetting of the Sm-Nd 'clock' means that the igneous event caused the efficient separation of a light rare earth element-enriched liquid from a crystalline residue. This is unlikely to have been achieved by transient heating following impact and hence points to volcanism on a planetary body only 1.24 Gyr ago. The environment was oxidizing and hydrous¹⁰, so Mars is a possibility. However, the absence of shock features in the Nakhla renders it unlikely that they could have been accelerated to the escape velocity of Mars, some 5 km s⁻¹. In contrast, the rare meteorites known as shergottites were shocked about 200 Myr ago, although their magmatic crystallization ages are somewhat older¹¹. Thus a martian origin is just conceivable for the shergottites.

Finally, let us turn to the origin of iron meteorites. About 86 per cent of irons belong to one of 12 chemical groups, the remainder being distributed among 'groupless' each comprising one to four members. Scott has argued that the chemical composition of an iron was determined first by nebular condensation to form the parent body, then by crystal-liquid fractionation. In all, some 60 parent planetesimals may be represented among our meteorite collections.

More recently, Scott¹² takes into consideration cosmic-ray exposure ages, cooling rates of the irons and the probability of sampling asteroids. The exposure ages¹³ indicate that iron meteorites were randomly released as small objects from their parent planets from 50 to 1,400 Myr ago, except for members of groups IIIAB and IVA, which were mostly released in two events 650 and 400 Myr ago respectively. Such events are probably rare, and so it seems unlikely that many asteroids could have been involved in major collisions which produced potential meteorites. One possibility considered by Scott is that some of the parent asteroids are composite, each being comprised of a number of smaller planetary bodies which had individually differentiated into metallic core and silicate mantle before incorporation into the larger body.

There is, in fact, evidence to suggest that this was the case. Axon and Bevan¹⁴ interpreted the structure of the Jamestown (IVA) iron as the result of a pre-terrestrial, crater-forming impact on an unknown planetesimal. The irons of group IVA often are shocked and they have a wide range of cooling rates. These observations would be consistent with a history of formation in one planetesimal followed by incorporation into a second, larger body.

So, from information derived from rare or unique meteorites, we can conclude that we may have samples from an ancient, lunar-sized object, from Mars, and from composite asteroids. □

Robert Hutchison is at the British Museum (Natural History), where he is in charge of the National Collection of Meteorites.

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Surprises from the *Glomar Challenger*

from Roger N. Anderson

OCEANOGRAPHY is still very much a profession of explorers, since planned experiments are as likely to produce unexpected results as not. Anyone who doubts this needs only look to the number of scientific discoveries made each year by accident. Not completely random accidents to be sure, but rather unexpected incidents bedeviling inquisitive souls pushing frontiers we really know very little about. An excellent recent example comes from the derrick floor of the deep-sea drilling ship *Glomar Challenger*. In March of this year, she was drilling into the pile of oceanic sediments being scraped off the American plate as it is being thrust deep beneath the Antilles volcanic arc north of Barbados in the western North Atlantic. Such subduction zones where one plate is forced deep into the mantle under another plate are sites of the primary collision events in plate tectonics. Yet we know little of the mechanics of plate interaction at such deep-sea trenches. The mission of the *Challenger*, led by co-chief scientists Casey Moore of the University of California at Santa Cruz and Bernard Biju-Duval of the Institut Français du Pétrole, was to drill through the newly accreted sediments plastered onto the Antilles arc and penetrate with the drillbit the subducting oceanic crust of the American plate. Determination of the mechanism of accretion was the underlying scientific objective. Little did they know the bizarre turn of events they would soon face.

Their first attempt (hole 541) turned up a major stratigraphic inversion with Lower Miocene (older) sediments overlying middle Pliocene (younger) sediments. They had indeed expected this result because newest sediments are pushed under

older muds, forcing the latter up and out of the ocean towards the Antilles volcanic arc (Fig. 1). Clearly, major overthrust events make up the accretionary prism of off-scraped sediments. The question is how are such large slices of sediment moved the many tens of kilometres along fault zones as required by this overthrusting mechanism.

Drilling conditions were rugged, however. Cave-ins and torqueing finally caused them to abandon the hole well above their oceanic crustal target. Undaunted, they offset 1-½ km towards the trench and tried again (at hole 542). Three times the hole caved in. The water depth was more than 5.5 km. Finally they tried a technological innovation never before used in the deep-sea — 'drill-in casing'. This casing has a drillbit at its tip. You drill as deeply as you can until the casing is stuck in the collapsing hole and you then unlatch the casing and re-enter the now walled-off hole with ordinary drill pipe. By drilling through the old drillbit, a new hole is begun. But the unlatching mechanism failed. The *Challenger* drillstring was stuck in the hole.

For 12 hours the drill crew worked feverishly to free the drillstring from the hole, finally succeeding by detonating dynamite inside the pipe and blowing free of the stuck casing. It was during these 12 hours that the unexpected happened. After they became stuck, no one much noticed the surge of water up the inside of the pipe as the first joint was broken at the rig floor. Being stuck is big trouble during any drilling operation. Yet a 'head' of about 6 inches of water was surging out of the drillpipe and onto the rig floor. It was only after blowing the charge downhole that the reverse flow stopped. A quick check of the derrick floor pressure monitor showed 350 p.s.i. of excess pressure at the surface. The stuck casing had formed a natural packer which acted as a stopper to prevent flow around the outside of the drillpipe to the sea floor. Thus the formation 6 km below, in which the casing was stuck, was connected directly through the inside of the drillpipe to the rig floor. The formation was overpressured — that is, its *in situ* pressure was greater than the hydrostatic weight of water inside the drillpipe. The overpressure pushed upwards on the water column forcing flow out onto the rig floor. Eventually, formation pore fluid actually flowed onto the rig floor.

The formation pressure was about 20

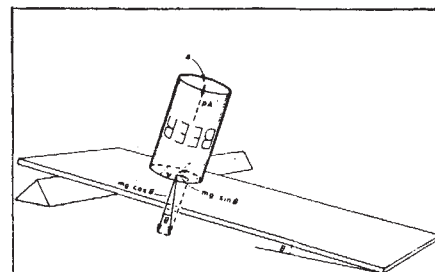


Fig. 2 To quote Hubbert and Rubey (*Bull. geol. Soc. Am.* 70, 121, 1959), an "elegant demonstration" of the effect of overpressures on frictional resistance to sliding comes from another famous geophysicist of the past, M.A. Biot. An empty beer can placed upside-down on a slightly inclined piece of plate glass will not slide. However, place an empty beer can directly from the freezer onto the glass upside-down and it will immediately slide to the edge of the glass and stop. To quote again, "The physical reason for this behavior is exactly the same as that which we have deduced for the case of overthrust faulting. As the cold can becomes warm, the air inside expands and causes the pressure to increase. This, in turn, partially supports the weight of the can and so reduces the normal component of force between the metal and the glass without affecting the tangential component. The can stops at the edge of the glass because the pressure is released".

bars above the hydrostatic or equilibrium pressure of 550 bars. That amounts to just about the weight of the overburden or lithostatic pressure at that depth in the drillhole. The importance of this becomes apparent when one realizes that the formation in which the drillstring had become stuck is the shear or fault zone between two massive wedges of sediment being overthrust onto the accretionary prism of the volcanic arc. The *Challenger's* scientists had stumbled upon a direct measure of the mechanism by which that overthrusting is occurring.

In order to understand this mechanism, we must go way back into the geological literature to one of the classic papers of the profession. In 1959, M. King Hubbert and William W. Rubey (*Bull. geol. Soc. Am.* 70; 121, 1959) wrote two companion papers explaining how great thicknesses of rock on land could be overthrust many tens of kilometres at very low thrust angles along fault planes only centimetres thick. Examples are the great overthrusts of south-west Wyoming, the Appalachians, and, classically, the Nappes of the Alps. Hubbert and Rubey proposed that excess pore pressures within a fault zone dramatically decreases the frictional resistance to sliding by reducing the critical

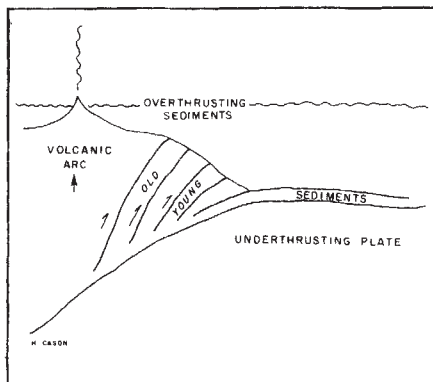


Fig. 1 Schematic diagram of an accretionary prism caused by sediments from the subducting plate being scraped off by the over-riding plate — in this case a volcanic arc. Accretion occurs as older sediments thrust over progressively younger sediments as the trench is approached.

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value of shear stress required to produce movement along a fault (Fig. 2).

The results of the *Challenger* 'flow-out' indicate that the large overthrusts of older sediment over newly scraped-off oceanic sediments landward of trenches occur along fault zones 'lubricated' by excess pore pressures. The *Challenger* also turned up evidence for the mode of origin of these overpressures. Temperature measurements made at depths of 50 to more than 150 m into the sediment above the fault zone gave values closely grouped at 14 to 16°C. These values are unusual in two respects: the temperatures from 50 to 150 m sub-bottom are virtually isothermal instead of increasing uniformly with depth and they are much hotter than the more typical 5 to 10°C for such tectonic settings.

Upward advection of warm pore fluids through a highly permeable sedimentary section is implied. The origin of this advecting water is likely to be the oceanic sedimentary section being squeezed during the subduction event. The newly released water not only advects upwards but also causes abnormal overpressures, allowing overthrusting to occur along well lubricated fault zones.

Finally, it is sobering to find the explanation of so fundamental a plate tectonic process in the literature of a previous time. Hubbert and Rubey (1959) themselves trace the origins of their solution to the problem back to the mid 1920s, and the field evidence for overthrusting began to accumulate even as early as the 1840s. □

Do radon anomalies predict earthquakes?

from Chi-Yu King

THE discovery that the radon content of groundwater in a deep well increased significantly before the magnitude 5.3 Tashkent earthquake in 1966 has prompted many research groups in the USSR, China, Japan and the US to monitor radon levels in wells and springs in earthquake-prone zones. Many radon anomalies have now been recorded, especially in Russia and China, and some are thought to be related and precursory to earthquakes as far away as several hundred kilometres^{2,3}. Such claims have often met with scepticism, particularly in the United States because no known mechanism could explain the relationship. Recently, a US research group reported a similar finding in southern California, a region considered to be a major seismic gap, where a long segment of the San Andreas fault was broken during a magnitude 8 earthquake in 1857 and where recent episodes of aseismic crustal uplift have been hotly debated⁴.

Shapiro and his colleagues at the California Institute of Technology reported⁵ that during the second half of 1979, anomalously high radon emanation was recorded at two of their three monitoring stations spaced about 30 km apart along an east-west frontal fault system of the Transverse Ranges of southern California. They suggested that the radon anomalies, together with several other anomalies recorded at about the

same time in and near the Transverse Ranges by other investigators, may be the result of large-scale strain and that they may have been precursory to a magnitude 6.6 earthquake on 15 October 1979, in the Imperial Valley, which is about 290 km to the south-east of the radon stations.

Shapiro also cites anomalies observed 20 km east of his eastern station at Arrowhead Hot Springs by Craig and co-workers⁶ of the Scripps Institution of Oceanography. The group took monthly water samples from Arrowhead and 15 other springs and thermal wells scattered throughout southern California between the Transverse Ranges and Imperial Valley. They analysed the contents of radon, helium and several other dissolved gases and found the values at Arrowhead to have increased significantly between May and the end of 1979. They attributed these increases to a smaller (magnitude 4.8) but closer (30 km) earthquake that occurred on 30 June at Big Bear Lake. No significant anomaly was recorded at any of the other 15 sites during this period.

A third group studying groundwater radon in southern California is headed by Teng⁷, of the University of Southern California. He analysed water samples taken once a week from 13 wells and springs in the Transverse Ranges. One of the study sites was Arrowhead Hot Springs, about 150 m from Craig's site, and a sudden increase in radon emanation was detected in May 1979. However, not enough data were collected at Arrowhead to see how long the radon anomaly lasted. Teng and colleagues detected, in addition,

some spiky radon increases at two other sites about 10 and 50 km, respectively, north-west of Arrowhead, and they consider the anomalies at all three sites to be precursory to the Big Bear earthquake.

Previously, radon anomalies had been observed before the magnitude 5.0 Malibu earthquake on 1 January 1979, at one of Teng's sites in the Malibu Mountains and at Shapiro's western station about 54 km east of the epicentre. However, no significant anomaly was observed before some other significant southern California earthquakes, notably the magnitude 5.6 Santa Barbara earthquake on 13 August 1978, about 150 km west of Shapiro's western station.

The recent radon data from southern California (like those from USSR, Japan and China), although encouraging, leave many questions unanswered, even if we assume that the anomalies are truly earthquake related and not merely coincidental. For example, why are some earthquakes preceded by radon anomalies and others not? Why have the 1979 anomalies been recorded mainly at scattered places in the Transverse Ranges, where no earthquake of magnitude larger than 5.0 occurred, yet in Imperial Valley, the site of the largest earthquake, no anomaly other than a 15-week drop in seismicity was recorded? If anomalies are site dependent, what factors distinguish a good site from a bad one? One might also ask whether the empirical relationships given in the literature between the amplitude, duration and spatial extent of the anomalies and the magnitude, time and location of the ensuing earthquakes are valid or oversimplified, why some radon anomalies are episodic, but others spiky, and why most anomalies are positive, yet a few negative (decrease from background level). Finally, what are the mechanisms that relate radon levels and earthquake activities?

Clearly more data on radon, as well as on other geochemical and geophysical parameters, are needed. During a recent trip to China, I was told that hydrogeochemical studies were accorded the third priority in the Chinese earthquake prediction programme, after seismicity and crustal deformation studies. Research is progressing rapidly in the United States and nearly 30 pertinent papers have been published during the past 2 years, most of them in two special issues of the *Journal of Geophysical Research* (June 1980) and the *Geophysical Research Letters* (May 1981).

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Vision and the optic flow field

from J.R. Lishman

ATTEMPTS to understand how animals see have largely been made by the study of the perception of stationary forms even though it is probably true that the greater part of our, or any other animals, use of the visual sense is in the control of movement. When we are walking, running, driving or catching a ball the visual stimuli we are receiving are constantly changing. There is perhaps a tendency to believe that we can comprehend such changing stimuli only by performing a sort of 'frame-by-frame' analysis which reduces the dynamic input to a series of static pictures and that an understanding of perception during motion will come only after we have understood picture perception.

A different research strategy is to look for relationships in the dynamic pattern of visual stimulation — the optic flow field — that might be used directly to control behaviour. An example of this approach is seen on page 293 of this issue of *Nature*. It is shown that the time at which a diving seabird closes its wings before hitting the surface of the water appears to be controlled by a visual variable (τ) which gives, at constant velocity, the time-to-contact with the surface of the water and which the bird could measure from the optic flow field without knowledge of either distance to the water or velocity. The simple derivation of this tau variable from the changing visual stimulation at the retina is explained in the box alongside.

The time-to-contact at constant velocity given by the tau variable and the rate of change in the time-to-contact during acceleration and deceleration given by the time derivative of the tau variable may be exploited to control behaviour in a variety of tasks. One that has been recently examined is the control of braking by car drivers¹.

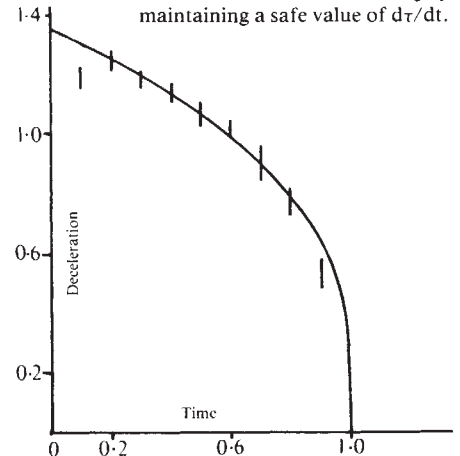
Consider someone driving along a straight stretch of road with another car ahead. If the lead vehicle is travelling at the same or a faster speed than the following vehicle then its retinal image size will be constant or contracting and if it is stopped or is travelling at a slower speed its image will be dilating. The change in the optic image of the lead vehicle tells the driver whether he is closing on the vehicle ahead. If he is closing on the vehicle then he has to begin braking early enough and control his deceleration appropriately to avoid an accident. At first sight it may seem that to do so the driver must know his velocity, the deceleration produced by his braking and his distance from the obstacle ahead. He can, however, control his braking force (and thus the rate of change of his own velocity) much more simply and directly through monitoring the rate of change of the time-to-contact with the lead vehicle (the time derivative of the tau variable).

As can be seen in the box below his current deceleration is adequate if and only if $d\tau/dt > -0.5$.

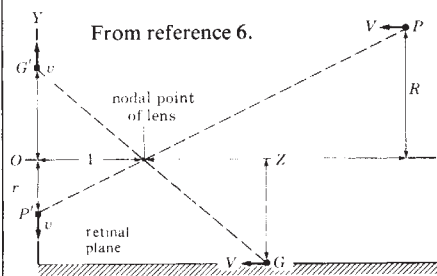
There is evidence that a driver does, in fact, control his braking by attempting to maintain a safe margin value of $d\tau/dt$. Spurr² found that deceleration curves of test drivers stopping at a fixed point tended to conform to the pattern of an initial steep linear increase in deceleration followed by a gradual decrease. If for an individual driver the deceleration curve for different speeds is plotted in a dimensionless form (that is, the deceleration as a fraction of the average deceleration throughout the stop against time as a fraction of the total stopping time) the curve is that expected if the driver had maintained a safe value of $d\tau/dt$ close to the critical value.

The agreement between curve and data is very close except for the initial build up of

The data are for a driver stopping at a nominated point from various speeds up to 100 km h⁻¹. The curve is what would have obtained if the driver controlled his braking by maintaining a safe value of $d\tau/dt$.



WHEN an animal is moving through the environment the optic array at the eye is constantly changing and gives rise to an *optic flow field* on the retina. Image elements flow out from the point towards which the animal is moving. A rigorous description of the optic flow field shows that it contains a great deal of information which could be used for controlling behaviour.



For convenience let us consider the point of observation to be stationary and the environment to be moving towards it at velocity V in the direction K to O (see figure). The image of the environment falls on the retinal projection plane. Two texture elements, P and G , in the environment will give rise to two images P' and G' on the retina which move out along radial flow lines emanating from O . From similar triangles:

$$\frac{Z}{R} = \frac{1}{r} \quad (1)$$

(Z and r are dependent on time t) and if we differentiate with respect to time we obtain

$$\frac{R}{V} = \frac{r^2}{v} \quad (2)$$

where V is the velocity of the texture element P and v the velocity of its corresponding retinal image P' . Eliminating R between (1) and (2)

$$\frac{Z}{V} = \frac{r}{v} \quad (3)$$

Equations (2) and (3) mean that the distance coordinates of all visible texture elements are specified within a scale factor V — the optic flow field thus gives information about the relative distances, sizes and orientations of surfaces and objects in the environment.

Time-to-contact

Of great interest is equation (3) which gives the time-to-contact with the texture elements Z/V . This is equal to the optic variable τ , tau (τ) which is essentially the distance apart of image elements on a surface divided by their rate of separation and gives us the sensation of an object 'looming up'. It gives us a *direct* measure of the time-to-contact with the surface at constant velocity.

$$\frac{Z}{V} = \frac{r}{v} = \tau \quad (4)$$

Control of deceleration

As described in the text the time-to-contact and its rate of change can be used by a car driver to control deceleration to avoid collision with an obstacle ahead. A driver travelling with velocity V and stopping with a deceleration D will come to a complete halt in the distance

$$\frac{V^2}{2D}$$

The driver will avoid a collision if this distance is less than that to the obstacle Z .

Thus the deceleration is adequate if

$$\frac{V^2}{2D} \leq Z \quad (5)$$

or

$$\frac{ZD}{V^2} \geq 0.5 \quad (6)$$

Now from (4)

$$\frac{Z}{V} = \tau$$

Differentiating with respect to time t

$$\frac{ZD}{V^2} = 1 + \frac{d\tau}{dt} \quad (7)$$

Combining (6) and (7) we see that

$$\frac{d\tau}{dt} \geq -0.5$$

that is, if the rate of change of the tau variable is -0.5 then the driver is decelerating rapidly enough to avoid a collision.

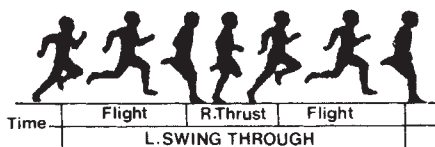
deceleration — this is to be expected as the theory simply assumes the driver instantaneously reaches a particular deceleration while a real driver does not 'slam on the brakes'.

The above argument is not restricted to the control of man-made machines: exactly the same kind of visual information could be used to control landing whether of a bird on a branch or a bee on a flower and there are numerous other skills which require accurate visual monitoring of time-to-contact.

Analysis of the optic flow field may enable us to explain how athletes get the kind of precise visual information they need to control their footing. One task that has been looked at from this viewpoint is the long jump³. A long jumper sprints some 40 metres and then leaps off a 20 centimetre wide take-off board. To achieve a successful jump the athlete must leap from as close as possible to the front edge of the board without overstepping it.

Film analysis of jumps made by three international standard athletes shows that the run up can be divided into two phases. In the 'run up' the athletes, although trying to use a stereotyped approach, inevitably build up positional errors as they sprint down the track. Three or four strides from the take-off they average standard errors in footfall position of more than 30 cm. They then 'zero in' and use visual information to adjust the final strides so that they hit the board with a standard error of less than ten cms (at a speed of around 22mph).

How do they visually regulate their final



strides? As shown in the figure a stride is composed of three segments which can be varied independently: the *thrust*, from the start of the stride to the point when the foot leaves the ground, which can be increased by lowering the hips; the *flight* the airborne distance travelled which can be modulated by changing the horizontal impulse (the 'drive') which affects the velocity of the athlete or the vertical impulse (the 'lift') which affects the time for which the athlete is in the air; and the *landing length*, how far ahead the foot touches the ground, which can be increased by stretching the leg forwards.

Analysis of films of the athletes suggests that they zero in on the board by regulating only the flight segment of the stride and that they do so by modulating the 'lift', which changes only the flight time, rather than the drive, which changes the velocity. The problem of striking the board may thus be usefully conceived of as one where the athlete makes a timing judgement rather than a distance judgement: the duration of the remaining strides is programmed just to fill the time remaining to reach the board — the necessary information being given in the time-to contact variable in the optic field.

The apparent simplicity with which some behaviours might be controlled by the optic

flow field has lead some researchers (in particular JJ Gibson who provided much of the original inspiration in this area) to suggest that the relation between perception and the optical input to the eye is in some sense 'direct' and is characterised by an 'immediate pick up of information'. Recent controversy (see ref.4 for an attack on Gibson's theory and replies) seems to have been confounded by a lack of any clear agreement on what is meant by 'direct perception'. Some (its critics) hold that it implies the nonsensical idea that perception can take place without anything going on at all in the head. Others say that it merely implies, in contrast to the view held by many who study visual illusions, that perception does not require an animal to make 'hypotheses' about the world from inadequate visual cues.

Whether or not there is any value in the idea of 'direct perception' there is little doubt that a shift in emphasis from the analysis of static visual arrays to a consideration of what an animal actually uses visual information for (as Gibson⁵ does in his 'Ecological Optics') is likely to help us to understand how animals see. □

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100 Years ago

The Mountain Nestor or Kea (Nestor notabilis). — Whatever may have formerly been thought to the contrary, there can be now no doubt that animals are continually changing their habits in order to suit themselves to the altered circumstances of their existence. A very familiar instance of this is that of the common swallow, which, in Europe at least, usually builds its nest in chimneys. But a much more striking and less laudable change of habit has of late years taken place in a New Zealand bird, of which we herewith give an illustration. Parrots, though varying much in the details of their diet, are generally considered to be altogether frugivorous. Fruit and seeds, and in certain special cases moss and honey, are, no doubt, their proper food. But since the introduction of the domestic sheep into New Zealand the Mountain Nestor, which was previously content with a modest repast of an entirely vegetable character, has developed a taste for mutton. Many instances have now been recorded of this bird attacking not only sick and dying sheep, but; it is alleged, even those that are strong and healthy, though we should hardly suppose that this parrot exists anywhere in sufficient numbers to be likely to do the flock-masters any serious injury.



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REVIEW ARTICLE

Aspects of plant genetic manipulation

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The use of molecular and somatic genetics in plant breeding should facilitate the selection and enhancement of the production of plants with desirable characteristics. However, such techniques are still very much at the experimental stage and several major drawbacks must be overcome before they can be applied to agricultural practice—but the effort should be well worthwhile.

THE present intense interest of plant breeders in adding molecular and somatic genetics to classical breeding techniques stems from the fact that these new techniques overcome the restriction on gene flow between organisms that is inherent in conventional breeding techniques. Thus, somatic cell fusion permits genomes of sexually incompatible species and genera to be brought together, while recombinant DNA techniques permit the transfer of individual genes even between prokaryotes and eukaryotes. The application of these techniques to plant cells will be the main subject of this review; the reader is referred elsewhere for accounts of supporting techniques, in particular plant cell culture¹ and the regeneration of plants that can be used in breeding programmes^{2,3}.

For a cell to be transformed stably, a foreign nucleotide sequence has to be introduced into it in such a way that the foreign genetic information can be expressed. Attempts to transform eukaryotic cells with foreign DNA have recently been increasingly successful, because of the development of markers of transformation, the availability of restriction endonucleases which enable duplex DNA to be cut into discrete fragments and the availability of sensitive techniques, such as DNA–DNA hybridization, to ascertain the presence of foreign DNA in the genome.

Early attempts at transformation of plant tissues and cells using isolated DNA⁴ have been critically reviewed elsewhere^{5–7}. Although there was some evidence that exogenous DNA could be taken up by seeds, seedlings and cultured cells, most of these early reports were probably accounted for by adsorption of DNA to cell walls and cell membranes. Furthermore, it was not clear whether the DNA that did enter the cell was integrated and replicated in the host genome. Some evidence favoured that conclusion; other evidence suggested that the exogenous DNA rapidly underwent degradation and re-utilization for endogenous DNA synthesis.

More recent studies on the transformation of plant tissue have concentrated on protoplasts which, because of the absence of a cell wall, behave similarly to cultured animal cells, and yeast spheroplasts, which have been successfully transformed^{8–11}. Exogenous DNA can be inserted into protoplasts by polycation-enhanced endocytosis¹² or fusion-like procedures¹³, or through liposomes¹⁴. Using such techniques it is, for example, possible to demonstrate that bacterial plasmid DNA can be recovered from the nuclei of cowpea and barley protoplasts^{15,16}.

A generalized strategy for plant cell transformation requires the construction of a genetic vehicle which is capable either of integrating into the plant genome or of independently replicating in the plant cell. Such a vehicle would, at the least, incorporate a selectable plant marker and a site for integration of a passenger sequence. If the vehicle was to be amplified in bacteria before transformation it would also contain a selectable bacterial marker and, preferably, the genetic information to assist its multiplication in the bacterial host. Techniques of cloning DNA sequences in bacteria are, of course, already well established¹⁷.

Selection

One of the major hurdles in developing transformation systems for plants similar to those for yeast^{8,9} and mammalian cells^{10,11} is the paucity of stable auxotrophs in higher plants¹⁸. Nitrate reductase-deficient lines of tobacco¹⁹ are one of the few such auxotrophs; *nia* mutant lines, selected for chlorate resistance, lack nitrate reductase apoenzyme activity and cannot grow with nitrate as the sole source of nitrogen. Protoplasts can be readily isolated from the leaf mesophyll cells of these auxotrophs and show high plating efficiency; cell colonies from the protoplasts regenerate into complete plants²⁰. This protoplast system should prove very useful for assessing transformation once cloned genomic DNA for nitrate reductase apoenzyme becomes available. Although it may be difficult to isolate this gene from plants it should be easier from fungi, such as *Aspergillus* and *Neurospora*. Many nitrate reductase mutants are known in *Aspergillus*²¹, from which genomic libraries have already been made²².

In the long term it is likely that positive selective markers, which can be added to a normal prototrophic genetic background, will be preferred. An ideal genetic marker would be a drug- or heavy metal-resistance gene. A large number of defined drug-resistance markers are known in prokaryotes²³, and recent reports show that these can be expressed in eukaryotes^{24,25}. Thus, when yeast spheroplasts lacking the *Leu*² gene were transformed with a chimaeric plasmid constructed from *Escherichia coli* plasmid pBR325, a prokaryotic gene for chloramphenicol acetyltransferase, yeast 2 μ m DNA and the yeast *Leu*² structural gene, yeast cells selected for *Leu*²⁺ prototrophy had chloramphenicol acetyltransferase activity and were resistant to chloramphenicol²⁴. Transformation of yeast spheroplasts to a state of resistance to the antibiotic gentamycin G418 has been achieved using the colicin E1 derivative pA043 plasmid carrying the transposable element Tn601, which renders bacteria resistant to the antibiotic²⁵. In both these cases transformation is unstable during mitosis on nonselective media because the DNA does not become integrated in the genome. Animal cells have been efficiently transformed to G418 resistance by part of the Tn5 transposon linked to a viral promoter²⁶.

Integration

There are two major experimental approaches to the integration of exogenous DNA into host plant cell genomes. The first is to attach sequences from plant DNA to the exogenous DNA so as to create sequence homology between host DNA and the vehicle for integration. One might, for example, use the tandemly repeated plant genes for ribosomal RNA²⁷, already cloned in bacteria, in an approach based on the observation that chimaeric plasmids containing yeast ribosomal DNA sequences integrate into yeast DNA and transform at high frequencies²⁸. Other sequences which may be useful for promoting integration into plant DNA are the transposable elements of maize. These move from one site to another and integrate at various sites by a recombination mechanism which does not apparently depend

on extensive homology of the sequences involved²⁹. The activity of the sucrose synthetase gene of maize is affected by a transposable element, and as sucrose synthetase cDNA has already been cloned²⁹, it should be possible to isolate genomic DNA containing the sucrose synthetase gene and to watch for transposable sequences adjacent to this gene.

The other major possibility is to use sequences from the tumour-inducing (Ti) plasmid of *Agrobacterium tumefaciens* to promote integration of foreign DNA into higher plant cells. The molecular basis of tumorigenesis involves the stable integration, maintenance and expression of part of the Ti plasmid, the T-DNA, in transformed cells³⁰⁻³⁴.

The first stable transformation of higher plants by isolated DNA involved the incubation of *Petunia* suspension cell protoplasts with Ti plasmids in the presence of poly-L-ornithine. Cell colonies were selected for the acquired tumorous characteristics of phytohormone independence, opine synthesis and multiplication when grafted to healthy host stem explants^{35,36}. There are also preliminary indications that tobacco suspension cell protoplasts can be transformed with Ti plasmid DNA using Ca^{2+} /high pH treatment³⁷, or polyethylene glycol (G. J. Wullems, personal communication). Transformation with liposome-entrapped Ti plasmid DNA is another possibility, as it results in colonies containing T-DNA in their genome (K. L. Giles and R. C. Nutter, personal communications) and with a higher transformation frequency (K. L. Giles, personal communication) than with poly-L-ornithine³⁵.

There are two strategies for using Ti plasmids as vehicles for carrying exogenous DNA into host cells. The first is to insert the exogenous genes into Ti plasmids by *in vitro* methods before transforming the host cells in the ways outlined above. Because of the size of the Ti plasmid (molecular weight 95–156 $\times 10^6$) and its multiple restriction sites, it may not be possible to reassemble a functional plasmid with foreign DNA integrated at the desired site³⁰. However, some regions of conserved DNA may be strong promoters for binding both eukaryotic and prokaryotic RNA polymerases³⁸ and could be used in non-*Agrobacterium*-based plasmid vehicles to obtain transcription of foreign DNA.

The second strategy is to use the normal infective process of *Agrobacterium* to introduce Ti plasmids, modified by *in vivo* recombination, into host plants (Fig. 1). For example, transposon Tn7, carrying resistance to streptomycin, spectinomycin and trimethoprim, and carried on the broad host range plasmid pRP4, has been experimentally transferred from *E. coli* to a nopaline strain (T37) *Agrobacterium*³⁹. One of the transconjugants selected for pTi and Tn7 markers was able to induce tumours on tobacco plants. These tumours failed to synthesize nopaline because of the insertion of Tn7 in the nopaline synthesis region on the right-hand border of the T-DNA. DNA-DNA hybridization showed that the complete Tn7 insert was present in the plant tumour genome, integrated at its original site in the T-DNA³⁹, but it remains to be seen whether bacterial genes carried on the transposon are transcribed and translated in tumour cells. Using *Agrobacterium* with a gene coding for phaseolin inserted into its T-DNA, recent studies have obtained transcription of bean globulin RNA in tissue cultures from sunflower tumours, but not its translation (T. C. Hall and J. D. Kemp, personal communication). A method developed for site-directed mutagenesis of *nif* genes of *Rhizobium meliloti*⁴⁰ could be used for the insertion of Tn5, or other transposons, into different regions of the T-DNA of *Agrobacterium*. Tumour cells produced by such *Agrobacteria* could then be screened for drug resistance and for the presence of Tn5 sequences.

There are several ways in which infectivity of *Agrobacterium* can be used to introduce foreign DNA sequences into plants (Fig. 1). The simplest is to make tumours on intact plants and then study the tumour tissue for the presence and expression of foreign sequences. Such tumour tissues may be highly chimaeric and may have to be cloned for isolating transformed cells. A much refined procedure for effective cloning of transformed cells is to infect cells cultured in suspension⁴¹ and cells newly

regenerated from protoplasts with intact *Agrobacterium*. Cells regenerated from protoplasts^{36,42} can be transformed by *Agrobacterium* at the high frequency of 1–3%, and protoplast-derived colonies can be screened for tumour characteristics or traits conferred by foreign DNA. These systems should be applicable to most of the dicotyledons for which cell cultures and protoplasts are available.

A further refinement will be to suppress the oncogenic properties of the bacterium while maintaining its infectivity. Shoots can be regenerated from many octopine and nopaline tumours, but fail to develop roots and these must be maintained by grafting to healthy plants. Shoots which arise spontaneously on nopaline tumours, and which are referred to as teratomata because of their abnormal morphology, will undergo phenotypic reversion to develop normally, flower and set seed, when maintained as scions⁴³. Deletions and transpositions in the T-DNA region of octopine Ti plasmids affect tumour morphology and shoot regeneration⁴⁴. There are indications that plants recovered from tobacco tumours induced by an octopine strain of *Agrobacterium* containing a deletion in the T-DNA are also capable of root formation (M. van Montagu, personal communication). In tumours which have lost their regenerative capacity, it is still possible to obtain expression of T-DNA genes at the plant level by the production of somatic hybrids between tumorous protoplasts and those of wild-type plants exhibiting regenerative capability⁴⁵ (Fig. 1).

It is desirable that any potential vehicle should be seed transmissible if the use of this new technology is not to be restricted to vegetatively propagated plants. Although leaf and flower petals from grafted phenotypic revertants of a nopaline teratoma BT37 contain T-DNA, the F_1 progeny tend to be morphologically normal, require phytohormones for growth *in vitro* and lack T-DNA. Haploids raised from anthers of grafted teratoma plants also lack T-DNA⁴⁶, and meiosis is thought either to cause or select for loss of the foreign DNA. However, some contrary evidence has also been reported. The nopaline teratoma BT37, when treated with kinetin, gives rise to normal-appearing shoots which form complete plants that lack tumorous traits and have lost most of their T-DNA except for the border fragments. The F_1 progeny of these plants also contain border fragments, indicating a persistence of the T-DNA sequences during meiosis⁴⁷. In another study, octopine-synthesizing shoots, produced from tumours that develop when cells regenerated from tobacco protoplasts are transformed with octopine strains of *Agrobacterium*, grow when grafted to healthy tobacco stocks. These scions produce flowers which, when fertilized with pollen from healthy wild-type tobacco, set viable seeds. The resultant F_1 progeny synthesize octopine, suggesting that the T-DNA goes through meiosis (G. J. Wullems, personal communication), although this will only be confirmed if the progeny plant DNA can be shown to contain T-DNA.

Monocotyledons, a group to which all the cereal crops belong, are not susceptible to crown gall disease at the whole plant level, perhaps because they lack the wound response typical of dicotyledons. It remains to be seen whether cells regenerated from protoplasts of such plants are amenable to transformation with intact *Agrobacterium*, or whether protoplasts of monocotyledons can be transformed by isolated Ti plasmids.

Transformation without integration

Attempts are being made to exploit plant DNA viruses to carry exogenous DNA into host cells, there to replicate independently of the host genome. Of the viruses, the double-stranded DNA caulimoviruses⁴⁸ of which cauliflower mosaic virus (CaMV) is the type member, and the single-stranded gemini viruses such as bean golden mosaic virus⁴⁹, are receiving most attention. Only a small amount of CaMV (5 μg) is needed to cause infectivity, and its particular attraction as a vehicle is that it spreads to all cells of the plant. There is a need to find a site in the viral genome where foreign DNA can be inserted in such a way that it does not interfere with viral replication and yet is actively expressed under viral control during normal infection. A possible insertion

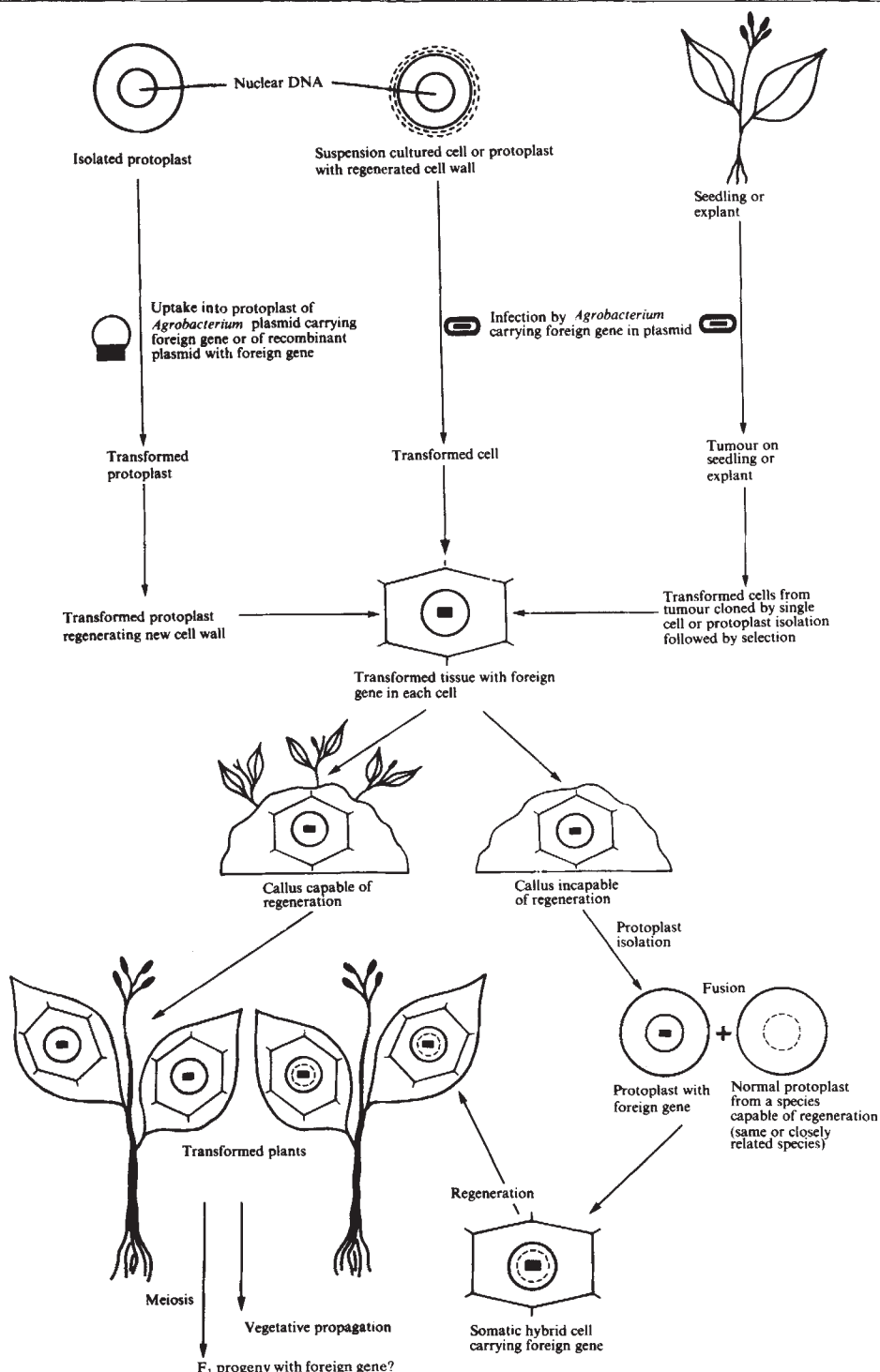


Fig. 1 Strategies for transformation in higher plants using *Agrobacterium*, *Agrobacterium* plasmid and recombinant plasmids. Three experimental systems are shown as alternative recipients for foreign genes depending on the vehicle used. If the capacity for plant regeneration is lost in the transformed callus, it could be restored following the fusion of the transformed protoplast with a normal protoplast of the same or different species.

point in CaMV is the site coding for the virion protein p66, which is the most abundant virus-encoded protein in the infected plant⁵⁰. However, it remains to be seen whether the small size of the CaMV genome will place too great a limit on the amount of foreign DNA that can be carried. Nor is it known whether the disease symptoms of CaMV infection can be suppressed. The stability of the CaMV DNA as an independent replicon needs to be studied further by infecting dividing protoplast systems with CaMV DNA.

Isolating plant genes

Attention is also being paid to the isolation of desired sequences from large nuclear genomes⁵¹ of higher plants. The general approach is to isolate mRNA⁵²⁻⁵⁴, to make copy DNA (cDNA) with reverse transcriptase and to use the cDNA to isolate structural genes from libraries of nuclear DNA⁵⁵.

cDNA has been synthesized for leghaemoglobin⁵³, the small subunit of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase⁵⁴ and a large number of storage proteins including zein⁵⁶ and bean seed G1⁵⁷. Both plasmids and λ phage-derived vehicles have been used to clone these cDNAs. Structural genes for bean globulin G1⁵⁸ and leghaemoglobin⁵⁹ isolated from the plant genome using cDNA probes have been found to contain introns, like many animal genes⁶⁰. The bean globulin G1 has three introns in the region that has been studied in detail⁵⁸. Plants must therefore possess a mechanism for splicing out introns; and bacteria, which lack such a mechanism, are unlikely to be able to decode plant nuclear genes. However, plant chloroplast genes can be expressed in bacteria, as has been shown by the expression in *E. coli* of maize and wheat genes for the large subunit of RuBP carboxylase/oxygenase⁶¹.

Why transform plants?

The most desirable manipulations of plants are those which would achieve higher plant productivity by additive effects to a protrophic background. Major factors affecting yield are carbon input, nitrogen input, assimilate partitioning and senescence⁶², together with stress conditions which include salinity, drought and disease. Enhanced photoproductivity is the most important factor^{62,63}. One way to enhance photosynthesis might be to modify the activity of RuBP carboxylase/oxygenase; several proposals have been made as to how its efficiency could be improved^{64,65}. One possibility would be to modify the enzyme so as to reduce its affinity for O₂, a change which may be achieved through site-directed mutagenesis of the cloned gene. A difficulty here is that the enzymatic site is on the large subunit of RuBP carboxylase/oxygenase⁶⁶, which is encoded by the chloroplast DNA. Therefore, it would be necessary to insert the mutated gene into the chloroplast, for which no technique is available.

It has been suggested that the large requirements of soybeans for nitrogen during seed fill exceed the capacity of the plant to supply nitrogen by the combination of biological N₂ fixation and uptake of fixed nitrogen⁶⁷. As nitrate reductase activity could be the rate-limiting step between nitrate and total protein⁶⁸, it will be worth trying to substitute the nitrate reductase gene of soybean with that of a plant with a more efficient enzyme.

Because genomic sequences for plant storage proteins which code for abundant mRNA can now be isolated, it may be possible to modify these genes⁶⁹ to increase their relative content of amino acids essential for human nutrition. However, amino acid changes in the proteins may change the conformation of the protein, and seed fill may be impaired; moreover, because storage protein genes are transcribed and translated in abundance at a particular stage in the life cycle of the plant⁷⁰, the modified gene will have to be subjected to the regime which controls the expression of a battery of storage protein genes.

Given an effective vehicle, it may eventually be possible to transfer the numerous genes controlling nitrogen fixation (*nif*) from bacteria to non-leguminous plants and thus to enable the latter to grow independently of nitrogenous fertilizers. Cloning and amplification of the *nif* genes have eased the unravelling of the molecular organization of the *nif* gene cluster of *Klebsiella pneumoniae*^{71,72} and its transcription^{73,74}. Transfer and expression of *nif* in other microorganisms such as *Azotobacter vinelandii*, *Rhizobium* spp. *E. coli* and *Salmonella* has also been demonstrated⁷⁵. However, as has been emphasized, "though it would seem possible in principle to transfer *nif* genes to a eukaryote the possibility of their being expressed therein is remote indeed"⁷⁶. Recently, the *nif* gene cluster from *K. pneumoniae* has been transferred into yeast, but as yet no expression has been observed⁷⁷. The main reasons for this are the complexity of the *nif* cluster possessing internal prokaryotic promoters which may not bind eukaryotic polymerases, and the extreme sensitivity of nitrogenase to oxygen inhibition. It has been suggested that the latter problem could be resolved by constructing a suitable anaerobic habitat inside plant cells in which nitrogen fixation could occur. This environment could be provided by special mutant chloroplasts which fail to generate oxygen⁷¹.

An alternative way of conferring nitrogen-fixation capability to plants is to transfer structural genes of legumes involved in the symbiotic nitrogen-fixing association with *Rhizobium* to non-legumes. One such gene, that for leghaemoglobin, has already been cloned⁵⁹ and attempts are being made to make cDNA for other 'nodule-specific' genes which produce moderately abundant mRNA⁷⁸. Such probes could be used to isolate genomic sequences which could then be introduced into non-legumes using suitable vehicles. Gene transfer from legumes to non-legumes may also be possible by somatic cell hybridization.

Somatic cell fusions

Fusion of protoplasts of widely divergent incompatible species is known to result in the directional elimination of one of the parental genomes but sometimes with the retention of reconstructed chromosomes⁷⁹ and a few genes⁸⁰. Recent studies have indicated that, as with mammalian cells⁸¹, it may be possible to manipulate plant cells in culture by X-ray irradiation of the parental cells and other chromosome-destabilizing procedures so that directional chromosome elimination occurs after fusion^{82,83}. Additional methods for transferring only part of the plant genome between diverse species are highly desirable, as it is often advantageous to incorporate some limited genetic attribute of a donor species. Purified mammalian metaphase chromosomes are extensively used as vectors⁸⁴ and polyethylene glycol treatment has recently been used to induce the uptake of isolated metaphase chromosomes into recipient wheat, parsley and maize protoplasts⁸⁵. The demonstration that pollination of *Nicotiana* with highly irradiated, compatible pollen can cause a few genes from the pollen to be transferred to the egg without proper fertilization^{86,87} has stimulated great interest. Moreover, parthenogenetic plants are thereby produced, avoiding the problems of regenerating whole plants from protoplasts. The use of highly irradiated protoplasts fused with normal protoplasts may also facilitate the transfer of a few genes into a suitable recipient species. It has been suggested that such somatic transformation could be used to transfer nitrate reductase genes between sexually incompatible species provided suitable auxotrophic mutants capable of regeneration into whole plants are available⁸⁸.

Results obtained so far on the consequences of fusion of protoplasts have provided good evidence that, in many instances, fusion between those from sexually compatible species will result in the formation of heterokaryons in which both nuclear genomes are capable of forming stable nuclear hybrids. Organogenesis from hybrid callus will then result in the regeneration of somatic hybrid plants with both sets of parental chromosomes^{89,90}. Somatic hybridization can also be used to bypass incompatibilities that may exist between plant species. Hybrid plants have been produced between species that are difficult or impossible to hybridize conventionally^{91,92}, including the somatic hybrid between two sexually incompatible *Petunia* species which promises to be of horticultural interest⁹³.

Amphiploids that occur in nature are usually fertile, thus ensuring their long-term survival. Artificially induced amphiploids such as *Triticale* vary from complete fertility to complete sterility and so it is difficult to generalize about the future breeding behaviour of somatic interspecific and intergeneric hybrids⁹⁴. Apart from the problems of breeding behaviour, it is evident that progress in the application of somatic genetics to plants is being impeded by the fact that regeneration from protoplasts is at present restricted to a relatively few genera, often those of lesser economic value. Most of the selection methods described thus far, with the exception of individual heterokaryon culture, tend to lead to a preferential recovery of amphiploid somatic hybrids, or hybrids approximating to this, to the exclusion of other potentially very useful plant types. Those potential plant hybrids that may possess one complete genome with only a few chromosomes of the other parent may be lost during the process of selection, as they may not survive the selective growth conditions, or may not even be recognized as hybrids.

The cytoplasmic mix obtained from protoplast fusions is novel. It seems likely that, although it is relatively simple to bring together two plastid genotypes in a common cytoplasm by protoplast fusion, it is difficult to keep them together. The inevitable result will be the eventual somatic segregation of the two types into two cytoplasmic subspecies, each with only one of the two plastid genotypes in its tissues^{95,96}; and it has been suggested that the use of an albino mutant for selection may cause unidirectional segregation in the case of sexually incompatible species⁹⁷. Opportunities for increasing cytoplas-

mic variability by protoplast fusions may be greater with mitochondria than with chloroplasts because there is evidence for mitochondrial recombination in cytoplasmic hybrids of *Nicotiana tabacum* by protoplast fusion⁹⁸.

If fusion of protoplasts is coupled with inactivation, by irradiation for example, of the nuclear genome of one of the species, and suitable selection procedures are used, novel cybrids can be obtained. Transfer of cytoplasmic male sterility in *N. tabacum* (*Nicotiana suaveolens* cytoplasm) to *Nicotiana glauca* by protoplast fusion has been reported⁹⁹, as has transfer of cytoplasmic male sterility by protoplast fusion in the petunia¹⁰⁰. As an alternative to the inactivation of nuclei by irradiation, protoplasts can be fractionated into enucleate subprotoplasts⁸⁸ or microprotoplasts¹⁰¹ which may provide suitable nucleus-free fusion partners.

Prospects

The procedures of gene cloning will enable us to understand the genomic organization of higher plants and to isolate gene sequences important in plant productivity. These will be used to transform plant cells if there has also been the development of efficient vehicles which carry markers for selection in a normal prototrophic background. Transformation of plants will require an ability to regenerate from these plant cells, and during the next decade it is likely that regeneration of plants from protoplasts of the major crops will have been accomplished. In the

short term, recombinant DNA technology may be used to improve the practical performance of symbiotic nitrogen-fixing bacteria, and possibly to create new associations between prokaryotes and higher plants^{102,103}. However, many of the transformations of plants which would achieve increased plant productivity are quantitative rather than qualitative, and are controlled by polygenic complexes which are likely to be much more difficult to transfer.

Although it seems likely that plant breeding using well established conventional methods will continue to make the key contribution to meet the demands of increased food production¹⁰⁴, multiple gene transfer mediated by somatic hybridization will, during the next few decades, assume increasing significance in the improvement of forage legumes, vegetables and fruit crops. Steps towards a broader spectrum of interspecies hybridizations in horticulture have already been taken⁹³. During the next decade it may be advantageous to attempt to incorporate into the recipient crop species some limited, perhaps single, genetic attribute of a donor species using a transformation procedure which will eliminate the need for backcrossing and recurrent selection¹⁰⁵. In the more immediate short term, practical gains will be made using tissue culture and protoplast cloning to enhance and release somatic variability in plants⁸⁸.

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ARTICLE

Fast atom bombardment of solids as an ion source in mass spectrometry

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A new ion source for molecular structure determination of thermolabile and involatile compounds by mass spectrometry is presented together with some preliminary results from peptides, glycoside antibiotics, organometallics and vitamin B₁₂ and its coenzyme.

THERE have been several attempts to overcome the necessity of presenting the sample to the mass spectrometer in the gas phase, before ionization. This limits the range of applicability of the technique due to thermal lability and involatility of numerous types of compounds, especially in the case of high molecular weight substances of biological and biomedical importance. This deficiency has been overcome by field desorption of ions¹, sputtering of ions from surfaces by bombardment with fast ions², and with heavy fission products from the radioactive decay of ²⁵²Cf (ref. 3) and the use of lasers to desorb ions from surfaces⁴.

Most of these methods have serious deficiencies, however. Field desorption is a difficult technique experimentally, necessitating the conditioning of fragile emitters, producing highly transient mass spectra, which in many cases seem to be dominated by thermal degradation of the material. The ²⁵²Cf plasma desorption source, although producing spectacular results, presents many difficulties, while laser-induced desorption of ions is in its infancy.

Perhaps the most tractable method has been to use the sputtering of ions by fast ion bombardment (SIMS)^{2,5} to produce both positive and negative ion mass spectra of small molecules. Again, there are several difficulties: (1) the ions produced have large kinetic energy spreads⁶, thus limiting the resolution available in simple mass spectrometers, and (2) the use of fast ions as the sputtering medium induces surface charging of insulating materials, and also introduces problems if the beam is to be steered into the high voltage regions of the ion sources of modern large mass spectrometers.

We have largely overcome problem (2) by using a beam of fast neutral atoms as our primary sputtering medium.

Experimental method

Our apparatus consists of a cold cathode discharge ion source producing Ar⁺ of controllable energy between 1 and 8 keV. The emergent beam is focused and passes through a collision chamber containing a high pressure (10⁻³-10⁻⁴ torr) of Ar gas. Resonant charge exchange occurs with little or no loss in forward momentum. This results in a beam emanating from the chamber which consists of a mixture of Ar atoms with approxi-

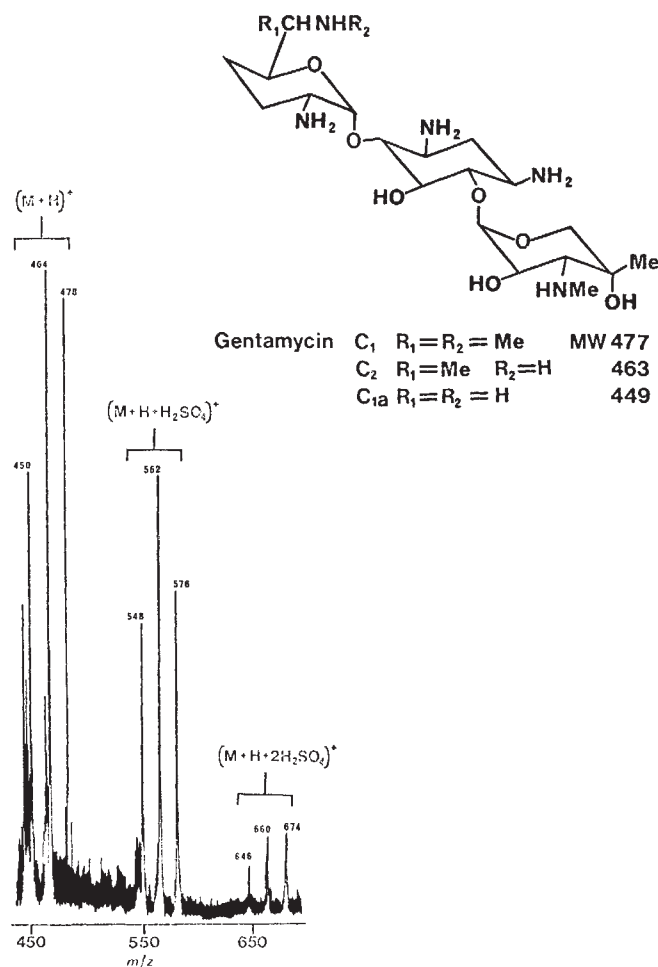


Fig. 1 Positive ion FAB mass spectra of a glycoside antibiotic: pseudomolecular and sulphate adduct ions of the gentamycins. MW, molecular weight.

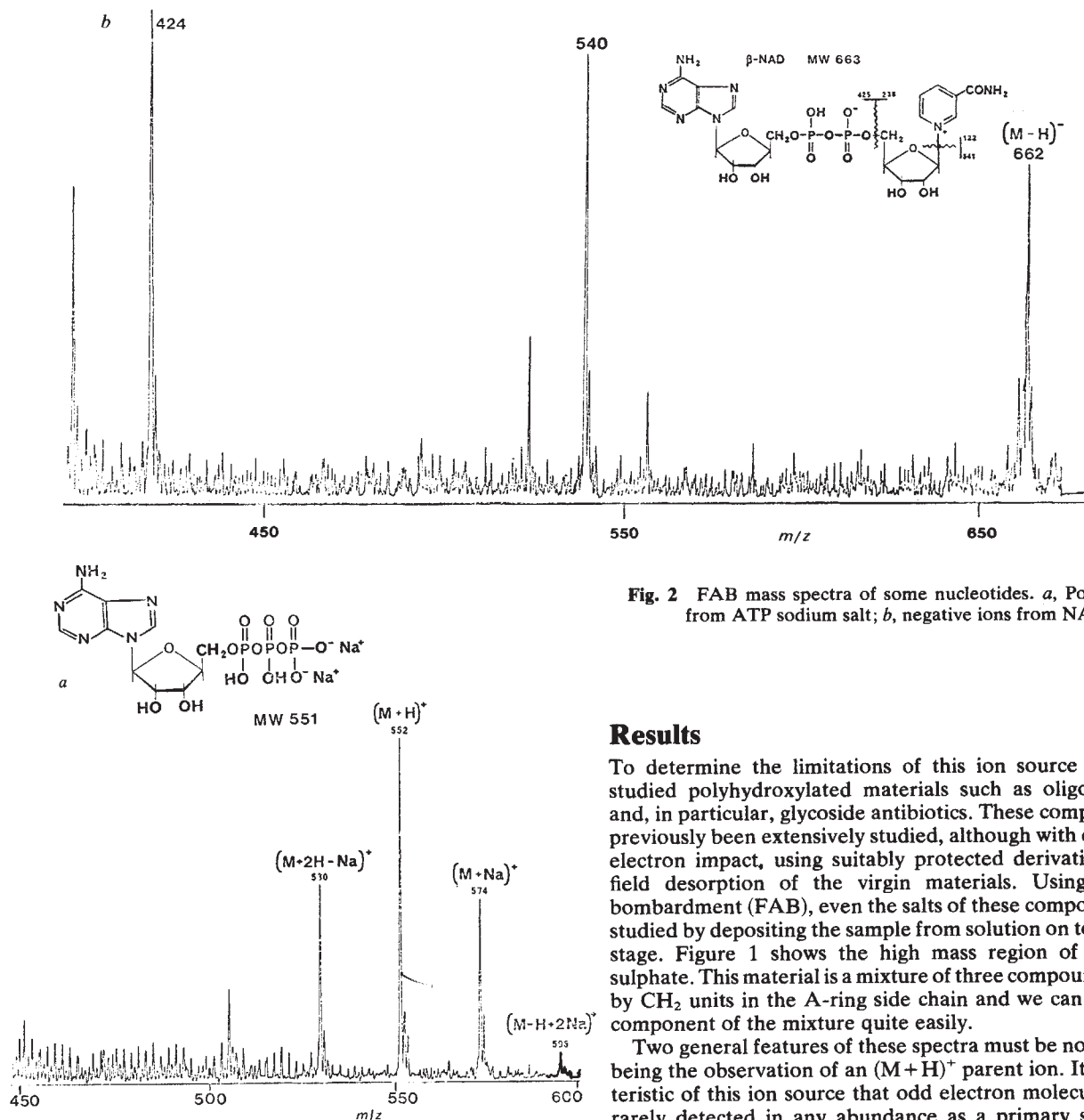


Fig. 2 FAB mass spectra of some nucleotides. *a*, Positive ions from ATP sodium salt; *b*, negative ions from NAD.

Results

To determine the limitations of this ion source we initially studied polyhydroxylated materials such as oligosaccharides and, in particular, glycoside antibiotics. These compounds have previously been extensively studied, although with difficulty, by electron impact, using suitably protected derivatives, and by field desorption of the virgin materials. Using fast atom bombardment (FAB), even the salts of these compounds can be studied by depositing the sample from solution on to the copper stage. Figure 1 shows the high mass region of gentamycin sulphate. This material is a mixture of three compounds differing by CH_2 units in the A-ring side chain and we can detect each component of the mixture quite easily.

Two general features of these spectra must be noted, the first being the observation of an $(M+H)^+$ parent ion. It is a characteristic of this ion source that odd electron molecular ions are rarely detected in any abundance as a primary species. The analogy may be drawn to chemical ionization in that as a rule, in positive ion FAB, even electron $(M+H)^+$ ions are produced, and $(M-H)^-$ in the negative ion mode. The second feature is that high sensitivity is obtained for such a low primary sputtering beam intensity. The intensity of the pseudomolecular ion peaks of the glycosidic antibiotic gentamycin is of the order of 10^6 ions s^{-1} .

Commercial samples of these materials are often in the form of sesquisulphates or hydrochlorides which have proved to be almost impossible to characterize by any other method in mass spectrometry.

Next let us consider simple mono- and dinucleotides. The mononucleotides have been investigated by making their trimethyl silyl (TMS) derivatives and using electron impact ionization. Some field desorption work has also been reported on the underivatized monophosphates⁸. There seems little problem in producing mass spectra from these molecules in an underivatized form using the FAB ion source, either as the free acids or as their sodium salts. Figure 2*a* shows the FAB spectrum of the sodium salt of ATP. We have also obtained spectra of similar quality from the dinucleotide salts of NAD and FAD, the former being illustrated in Fig. 2*b*. The fragmentation obtained from these molecules is shown and reveals considerable structural information.

mately the same kinetic energy as the original Ar^+ , and Ar^+ which has not been charge exchanged. The latter is cleansed from the beam by a set of electrostatic deflector plates. The whole system is differentially pumped to maintain an adequate vacuum in the mass spectrometer ion source. To minimize the surface damage, the intensity of the pure Ar atom beam is maintained at $\sim 10^{10}$ – 10^{11} atoms per cm^2 of target per s.

To help alleviate problem (1) we have modified the ion source region of an AEI MS902 double focusing mass spectrometer to accept our atom gun. The system has been adapted so that materials can be introduced by deposition from solution on to a copper stage outside the vacuum and inserted through a standard vacuum lock system into the MS902 source to intercept the fast atom beam. The ions produced by the sputtering mechanism are then accelerated to the normal spectrometer potential and pass into the analyser region of the instrument. With such a system we have produced moderate resolution mass spectra from a wide variety of molecules which have previously proved difficult. Using the double focusing instrument we have also carried out accurate mass measurements, and using the Barber-Elliott technique⁷ have observed first-field free region metastable transitions of ions produced by the sputtering phenomenon.

Another class of compounds, the sulphonic acid derivatives of aromatic naphthalene and anthraquinone systems, are of interest in the dyestuffs industry and have been extensively investigated using field desorption mass spectrometry which has proved to be of little value. We have obtained good spectra from molecules containing up to five sulphonic acid groups, as both free acids or their sodium and potassium salts. Figure 3 shows a typical spectrum which can be obtained from these compounds; considerable fragmentation of the molecule takes place which is

easily explained on the basis of the structure of the original material.

The ability to sequence peptides has probably been one of the most spectacular successes in structural mass spectrometry. Careful choice of derivatives were necessary when using conventional methods of ionization. The existing techniques have gained increasing popularity, and have led to the sequencing of not only moderate-sized molecules such as the enkephalins⁹, but, after suitable enzymatic cleavages, to the amino acid

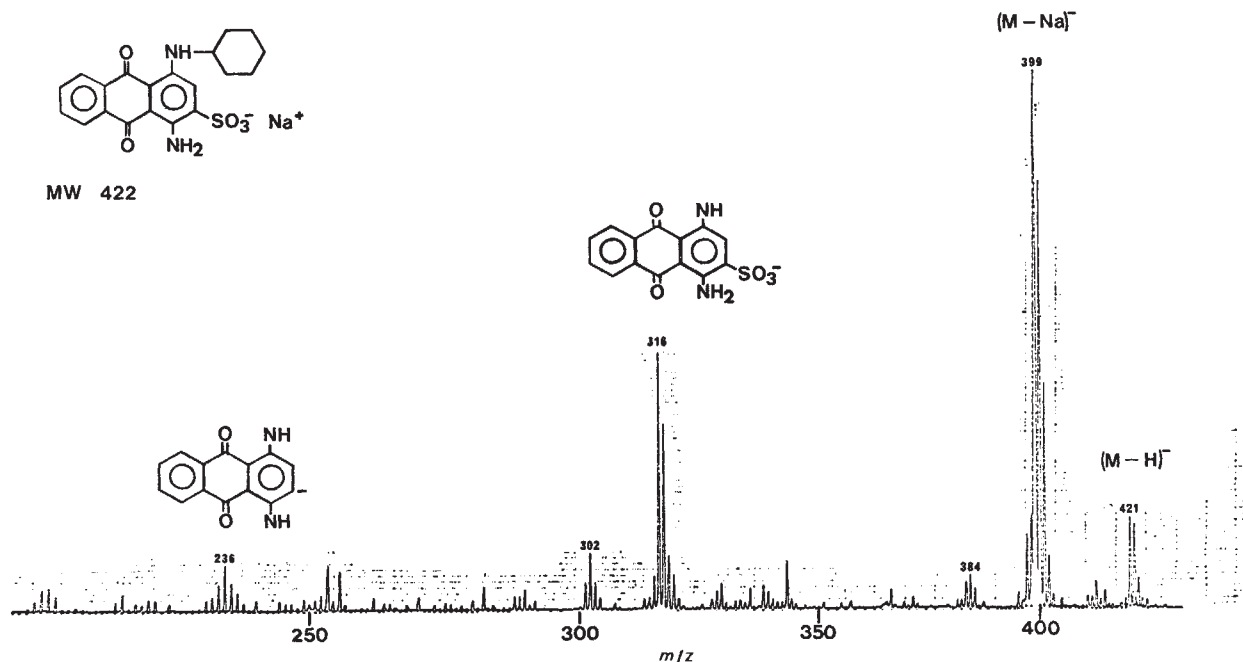


Fig. 3 Negative ion FAB mass spectrum of anthraquinone disulphonic acid.

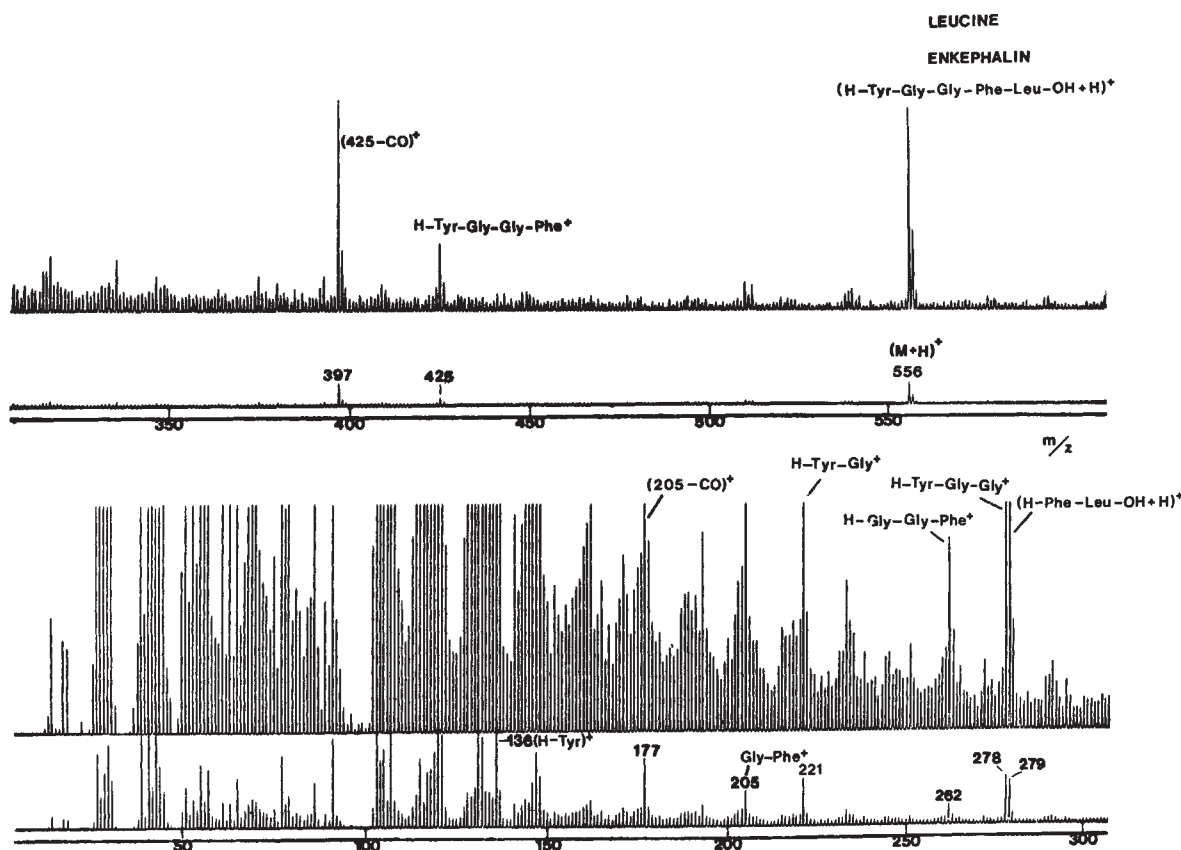


Fig. 4 Positive ion FAB mass spectra of the oligopeptide leucine enkephalin.

sequence of some proteins¹⁰. We have concentrated on investigating the FAB mass spectra of underivatized peptides, with a view to determining the size of peptide amenable to this method and the sequence information contained within the spectra. Figure 4 shows the positive ion mass spectra of leucine enkephalin on which the sequence ions are indicated. Similarly for bradykinin, the FAB spectrum revealed comprehensive sequence information with cleavages being observed for both the C-terminal and N-terminal ends.

The largest underivatized peptide that we have investigated is human gastrin 1 of molecular weight 2,096. This peptide gave reasonable 'pseudo-molecular' ion intensity, $(M+Na)^+$ at m/z 2,119, but lack of sensitivity with our present equipment has prevented detailed examination of the fragmentation of this molecule. From these preliminary investigations it seems that sequencing underivatized peptides at least as large as gastrin 1 may be possible.

We have also studied the fragmentation pathways which give rise to the major peaks found in the FAB mass spectrum of small peptides as revealed by standard metastable detection methods. Figure 5a shows such 'metastable' transitions occurring in the positive ion FAB mass spectrum of the tripeptide, H-Ala-Leu-Gly-OH and the transitions are interpreted. Alternatively, the Barber-Elliott method⁷ of metastable scanning on the same molecule is shown in Fig. 5b with the interpretation of the various transitions interpreted. This sort of information is crucial for understanding the mass spectra from any class of compounds, and we believe that this is the first demonstration that sputter ion sources produce 'molecular' species with internal energy, sufficient to produce ion decompositions in the gas phase, entirely analogous to the other ionization methods.

We now consider some organometallic systems. The first example is a rhodium complex, the positive ion spectrum of which is shown in Fig. 6a. This is of interest for two reasons. First, the molecule is thermally labile and water sensitive, and gave no mass spectrum of any worth by conventional methods. Second, it is produced as an oil, demonstrating that the FAB ion source can accommodate liquids. It is also of interest that the oil

produced a 'non-fading' spectrum without modified sample preparation.

Our final examples of organometallic systems are the vitamin B₁₂ complexes. Schiebel and Schulten¹¹ describe B₁₂ as a 'milestone' in mass spectrometry. We have successfully obtained mass spectra not only from cyanocobalamin but also from the hydroxy and methyl derivatives. The FAB mass spectrum of cyanocobalamin is shown in Fig. 6b. It is interesting to compare our spectra with field desorption spectra. These are dominated by a set of peaks at m/z 914 and m/z 932, with the parent peaks being very small. In our opinion, the field desorption mass spectrum is dominated by thermal degradation which we do not observe. In addition to these cyanocobalamins, we have been able to obtain the first mass spectrum of the coenzyme of vitamin B₁₂ as shown in Fig. 6c. The interpretation of our spectra will be discussed elsewhere.

Discussion and conclusion

FAB mass spectrometry has immense possibilities for extending the mass range of materials accessible to mass spectrometric analysis, especially when the usual mass spectrometric facilities are available. However, certain points and generalities concerning the characteristics of this ion source need clarifying.

First, sample preparation and manipulation are essentially very simple. The material is deposited from solution or a slurry on to the sample stage, requiring no previous generation of delicate substrates, as in field desorption. During our work we have noted, as others have reported¹² that in many cases the spectra obtained are transient; rapid 'fading' ensues with a half life for the pseudomolecular ion of the order of a few minutes. The origin of this fading is obscure, being possibly a mixture of surface damage by the primary atom beam, and surface contamination by the residual gases from the relatively poor vacuum obtainable in the ion source region in most conventional large mass spectrometers.

We have, however, largely overcome this effect by judicious use of solvent and support systems, paying particular attention

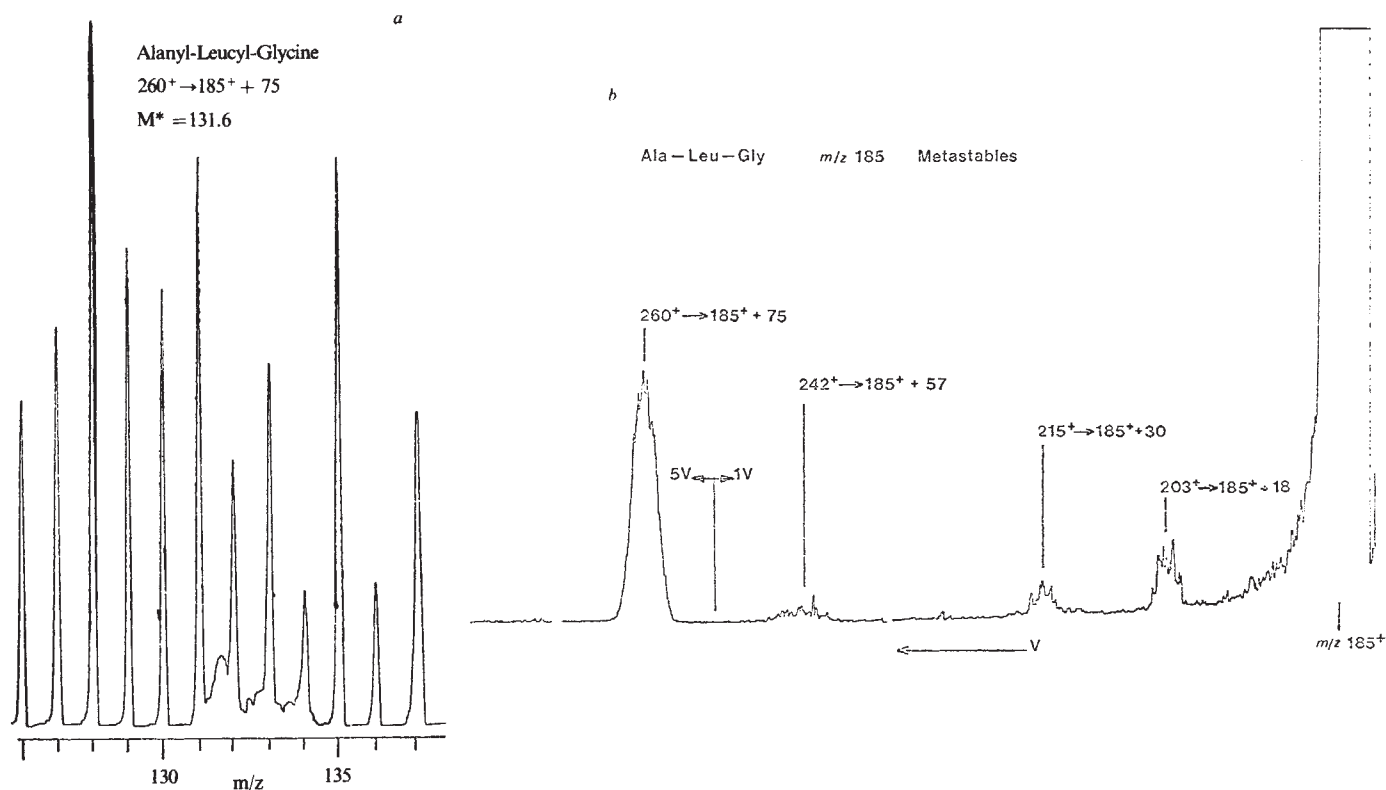
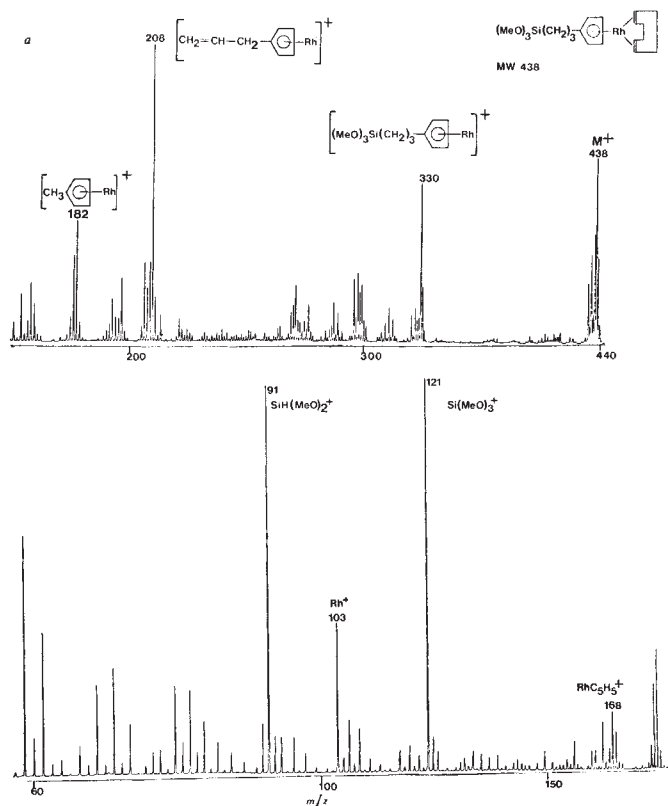


Fig. 5 Metastable ions in FAB mass spectra: a, second-field free region metastable ions in H-Ala-Leu-Gly-OH; b, Barber-Elliott metastable scan showing the precursor ions of m/z 185⁺ in H-Ala-Leu-Gly-OH.



Cyanocobalamin

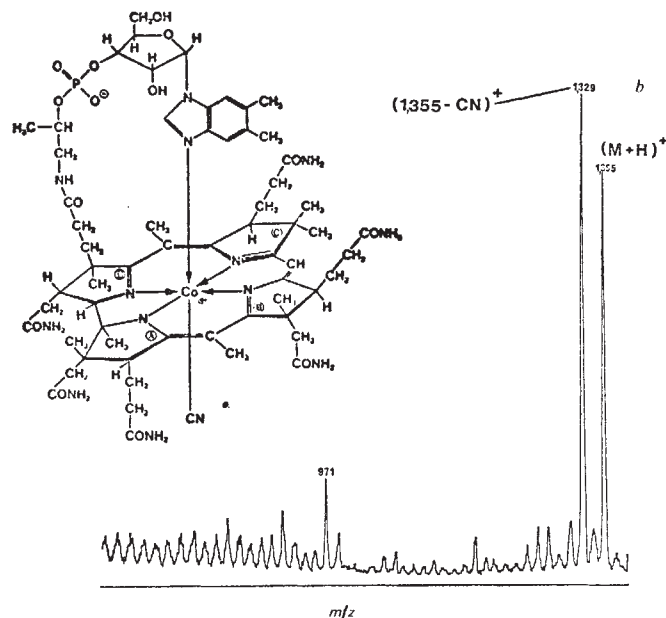
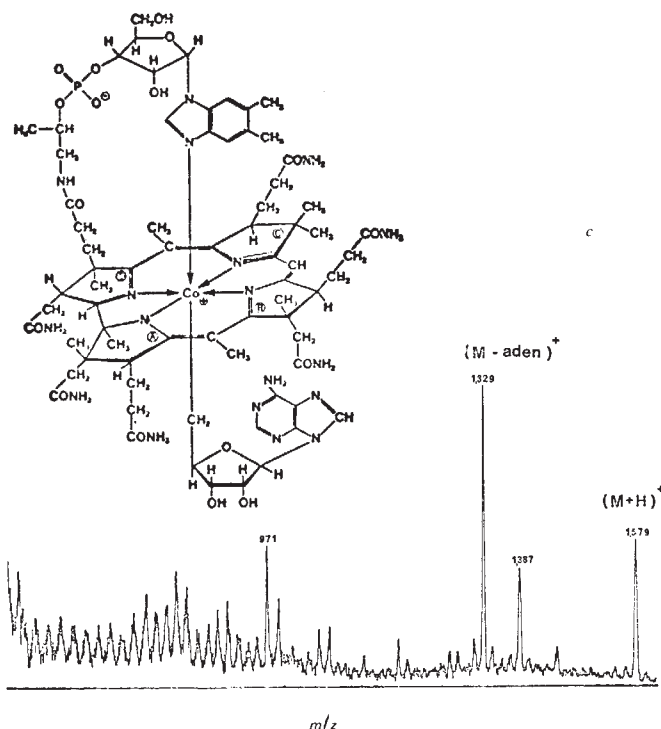
Coenzyme B₁₂

Fig. 6 Positive ion FAB mass spectra of some organometallic compounds: *a*, rhodium complex; *b*, cyanocobalamin (vitamin B₁₂); *c*, the coenzyme of vitamin B₁₂.

to the viscosity and volatility of the medium from which the sample is deposited on the stage. By this means we have preserved samples, with no depletion of 'parent' ion sensitivity over periods of hours.

One disadvantage of this ion source is that the ions produced have large kinetic energy distributions and most of the double focusing instruments obtainable commercially are not fully energy focusing. Indeed our instrument whilst performing fully first order focusing in terms of direction and energy, has a large second order energy aberration. If full use were to be made of this, and other high kinetic energy ion sources, then fully corrected second order focusing geometries must become standard in the next generation of high performance mass analysers.

Improvements must be made to the vacuum systems used in conventional organic instruments, such that ultimate pressures in the ion source and related regions of instruments should be in

the region of 10^{-8} torr or better. As sputter ionization methods are surface sensitive, they require an environment which makes surface contamination minimal.

We do not believe that we have yet nearly realized the full mass range capability of this ionization method. Indeed, using simple polymers as samples, we have covered the full mass range of our existing instrument (4,000 daltons) but this at a serious reduction in sensitivity due to the large reduction in ion accelerating voltage (a factor of 4) and consequent poor ion extraction efficiency from the source itself. High field strength magnets are available commercially, which partly alleviate this problem.

If, however, we consider a target specification as a mass range of 5,000–10,000 daltons at present source extraction efficiencies, then the physical size of the next generation of fully-energy-corrected instruments must be reconsidered.

A generalization of the ionization process, and other sputter sources, is that genuine odd-electron molecular ions are rarely observed.

Even-electron pseudomolecular ions of the type $(M+H)^+$ or $(M-H)^-$ are invariably observed. We have observed large M^+ ions in polynuclear aromatic systems, but metastable analysis has revealed that these arise by unimolecular dissociation of the $(M+H)^+$ species.

In many cases, especially for carbohydrates, we can stabilize the 'molecular' species by deliberate alkali ion addition to give an even-electron $(M+Na)^+$. Compounds presented to the source in the form of alkali metal salts, for example, carboxylic acid salts of the type $R.COONa$, give rise in the positive ion spectra to $(RCOONa_2)^+$. Such observations indicate that considerable ion-molecule interactions must take place during the collisional process of sputtering, and we may therefore draw some analogy to chemi-ionization as the predominant means of producing the ions we see. A simple, molecular dynamic treatment of the sputtering phenomenon¹³ suggests that the initial impact by the fast particles creates a growing cavity within the lattice which contains a high density gas of randomly distributed particles. A secondary effect due to the dendritic growth of collision chains within the lattice is considerable surface layer peeling, where whole layers of the material seem to be able to separate from the rest of the solid.

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Within the high density gas cavity, both positive and negative ions, neutral ions and electrons can be formed in the initial process by 'percussive' ion sputtering mechanisms. Here a molecule is placed on the high repulsive potential energy side of its potential energy hypersurface by the initial impact, and can then decay by surface crossings into dissociation products which are ion pairs or any other combination of ions, neutral species and electrons. We thus have a system in which a high probability exists that ion-molecule reactions occur in the high density cavity with subsequent chemi-ionization of neutral species which are being peeled from the surface. This, we believe, is the essence of the ionization processes occurring in this and other similar ion sources.

We conclude that the FAB ion source has the following advantages. (1) Ionization occurs from the solid, so no sample volatilization is necessary. (2) Sample preparation is easy compared with derivatization techniques or field desorption experiments. (3) There is high pseudomolecular ion sensitivity together with structurally significant fragmentation. (4) A very high mass range of molecules are now accessible.

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LETTERS TO NATURE

Hard X-ray spectrum of Cygnus X-1

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Cygnus X-1, a strong X-ray source in a binary star system, is generally considered a good candidate for a black hole¹. Its X-ray emission provides the best method of studying the physical processes near the collapsed object. The average energy spectrum (in the 'low' state) is remarkably stable² and has been interpreted as the result of Comptonization, that is Compton scattering of optical or UV photons in a very hot plasma³⁻⁵. We have measured the low-state spectrum on 26 October to 18 November 1977, over a wide energy range (3 keV-8 MeV). This is the first measurement to cover such a broad energy range and the first long-term average measurement above 300 keV. This spectrum agrees well with a single temperature Comptonization model at low energy, but shows a significant excess at high energy ($E > 300$ keV).

Our observations were made with two instruments on the HEAO 1 satellite, in the spacecraft's scanning mode, in which the X-ray detectors scanned great circles on the sky once every 30 min, covering the entire sky in six months. The lowest-energy data (3-40 keV) came from one of the xenon proportional counters of the GSFC Cosmic X-ray experiment⁶ (A2), during 3-8 November 1977. Higher-energy information (15 keV-

8 MeV) came from six different NaI scintillation counters in the UCSD/MIT Hard X-ray and Low-Energy Gamma-Ray experiment⁷ (A4). We obtained spectra from both of this experiment's low-energy detectors (15-180 keV), three of the four medium-energy detectors (100 keV-2 MeV), and the high-energy detector (200 keV-8 MeV) by a point-summation technique. Detector response functions were calculated for the proportional counter and the 15-180 keV scintillators by analytical models, and for the other scintillators by a Monte-Carlo simulation. Figure 1 shows the resulting composite spectrum for all detectors from both experiments. The proportional-counter spectrum has been multiplied by a factor of 1.3 to make the best match in the overlap range (15-40 keV). This is allowable because Cyg X-1 is variable and the proportional counter data were taken only during a small portion of the total observing time.

The strong X-ray source Cyg X-3 is only $\sim 8^\circ$ from Cyg X-1, close enough to confuse measurements made by detectors with wide fields of view. Fortunately, the 15-180 keV scintillators had a small field of view ($1.2^\circ \times 20^\circ$), thus we were able to obtain independent observations of Cyg X-1 and Cyg X-3 in this energy range. These showed that the emission of Cyg X-3 was negligible compared with that of Cyg X-1 above 100 keV. Thus we have rejected all data from the medium- and high-energy scintillators below 100 keV. The proportional counter also had a small field of view ($3^\circ \times 3^\circ$).

As Cyg X-1 is variable, we must show that the spectrum did not change during our observations. Figure 2 shows the history of the intensity of the Cyg X-1 flux, as measured by one of the 15-180 keV scintillators. There is no significant linear trend in these data. We compared the 15-180 keV spectra from 14 different two-day intervals, fitting each with a Comptonization spectrum. At the 95% confidence level, our measurements were consistent with no variation in the spectrum shape. Our 95% confidence limits for the r.m.s. variation in kT_e and γ are

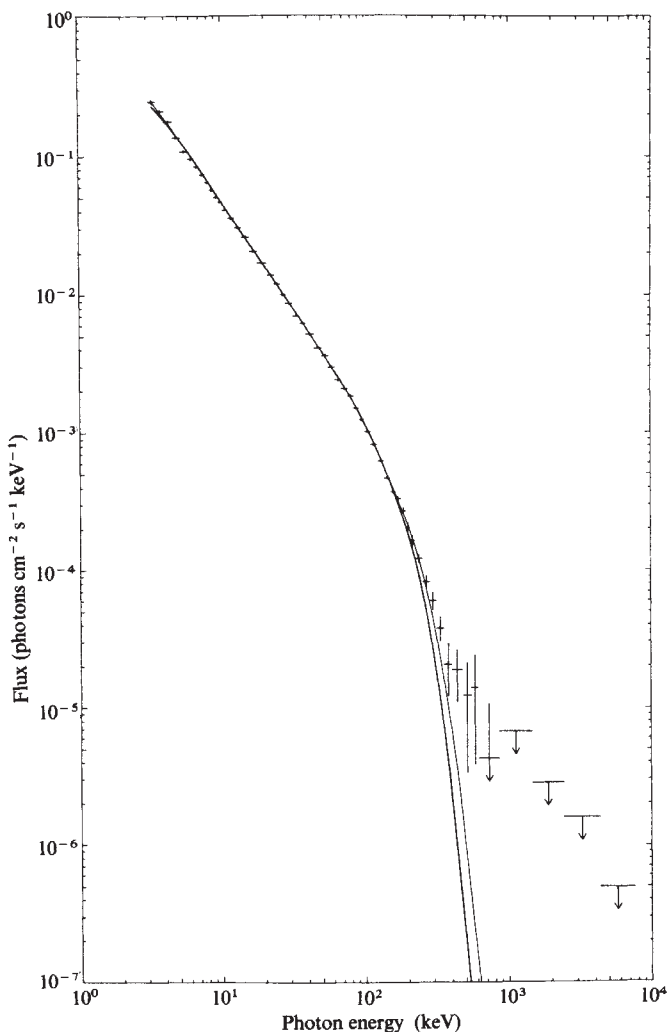


Fig. 1 Average photon spectrum of Cyg X-1 in October–November 1977. The upper limits are twice the statistical uncertainty. The heavy line is a single-temperature model which fits well below 200 keV, but falls far short of the data at higher energy. The light line is a two-temperature model which fits better.

$2.2^{+0.8}_{-2.2}$ keV and $0.07^{+0.03}_{-0.07}$, respectively. We also compared the 100 keV–2 MeV scintillator spectrum for the central 12 days of the observation (when there was a small increase in the low-energy scintillator rate) with the spectrum of the first and last six days. There was no significant difference up to at least 400 keV.

Figure 1 shows the best-fit model spectrum of the form described by Sunyaev and Titarchuk⁴ and by Sunyaev and Trümper⁵; this has the form

$$\frac{dN}{dE} = Ax^2 e^{-x} \int_0^\infty t^{n-1} e^{-t} \left(1 + \frac{t}{x}\right)^{n+3} dt \quad (1)$$

where A is a constant, $x = E/kT_e$,

$$n = -\frac{3}{2} + \left(\gamma + \frac{9}{4}\right)^{1/2} \quad (2)$$

$$\gamma = \frac{\pi^2 m_e c^2}{3kT_e \left(\tau_0 + \frac{2}{3}\right)^2} \quad (3)$$

T_e is the temperature of the electron gas, m_e is the mass of an electron, and τ_0 is the optical depth of the gas to Thompson scattering. This result is appropriate for a spherical gas cloud. For a disk geometry, which may be more appropriate for Cyg X-1, the factor 3 in the denominator of equation (3) should be changed to 12 (ref. 4). The best-fit spectrum has $kT_e = 32.4 \pm 0.4$ keV and $\gamma = 2.39 \pm 0.01$, with absorption by a hydro-

gen column density of $1.8 \times 10^{21} \text{ cm}^{-2}$. This gives an optical depth of 3.9 or 1.6 for spherical or disk geometry, respectively.

Our measurement below 200 keV agrees well (except for 30% greater intensity) with the most precise previously-reported spectrum⁵, which was fitted by a comptonization model with a slightly different temperature (27 keV) and optical depth (5). At higher energy (> 300 keV), we agree with most previous measurements^{8–10}, but below that of Baker *et al.*¹¹. However, our broad energy range and good low-energy sensitivity allow us to test the model more severely than do these previous measurements. Note that, in Fig. 1, the model function is a good fit to the data below 200 keV, but at higher energy, the data are in excess of the model. The excess contains about 5–10% of the total X-ray luminosity.

To account for the high-energy excess, we added a second component to our model spectrum, using as candidates a power law, a broad line at 511 keV, and a second comptonization spectrum with a lower temperature. Each of these diminished χ^2 for the fit significantly. An E^{-1} power law caused the reduced χ^2 to drop from 2.89 to 2.77 (for ~ 150 d.f.), which is significant at the 99.4% confidence level. A line at 511 keV with a width of 640 keV FWHM and a strength of 1.8×10^{-2} photons $\text{cm}^{-2} \text{ s}^{-1}$ produced a χ^2 of 2.57. Best by far was the second comptonization spectrum, with a χ^2 of 1.82. The two components had temperatures of 15.2 ± 1.0 keV and 41.6 ± 1.2 keV, and γ s of 2.4 ± 0.3 and 2.1 ± 0.2 (see Fig. 1). None of the models tried gave an acceptable value for χ^2 . Most of the excess χ^2 , however, comes from the energy range 15–40 keV, and might be attributed to systematic difficulties in combining data from two different instruments at the ends of their energy ranges. Small discrepancies in this energy range receive a very heavy weight because the statistical uncertainties are small. Note that the second comptonization spectrum reduces the significance of the excess flux in the 281–863 keV band only from 6.6 to 3.5 σ . There is still room for improvement at the high-energy end, perhaps in the form of a third component.

In the context of comptonization models, a distribution of electron temperatures from 15 to ~ 100 keV could explain the observed strong high-energy emission. The lower temperature limit for this distribution could not be much below 15 keV, as the single-temperature model fits fairly well at low energy. According to the two-temperature accretion disk model of Shapiro *et al.*³ (see their Fig. 4), we should expect just such a distribution, with a sharp lower-temperature cutoff.

Cyg X-1 is almost unique among galactic X-ray sources because of its hard spectrum at energies > 50 keV. A thermal

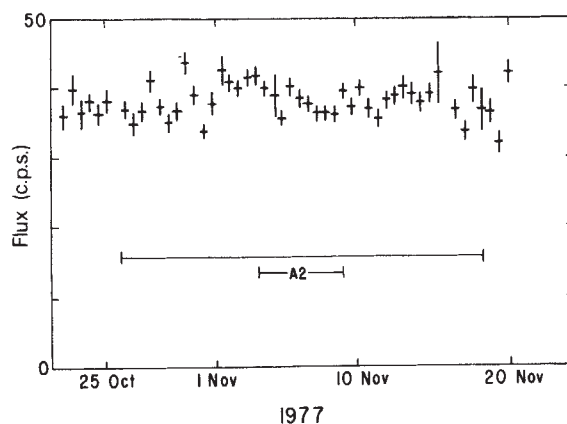


Fig. 2 Intensity of Cyg X-1 emission expressed as detector counting rate in the 15–180 keV band. Each data point represents 0.56 days, one tenth of the binary orbital period. The error bars represent 1σ uncertainties based on photon-counting statistics. There is considerable day-to-day scatter, but no long term trend. The long horizontal bar indicates the period in which the scintillation counter spectrum (15 keV–8 MeV) was measured, and the shorter bar 'A2' shows the time when the proportional counter spectrum (3–40 keV) was measured.

emission process, such as the Comptonization model discussed, would favour the identification of Cyg X-1 with a black hole or other object without a strong magnetic field, which would modify the spectrum through cyclotron processes. The spectrum of a thermal process would be cut off at an energy determined by the maximum temperature. Our observations show that such a maximum temperature must be at least in the neighbourhood of 50–100 keV, approximately the largest value allowed by current theory¹².

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Can the twin-exhaust model explain radio jets?

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The distinctions between compact cores in various powerful extragalactic sources have proved difficult to interpret. In a few source categories (for example radio galaxies and quasistellar radio sources) extended structure on much larger scales exist; their energy is undoubtedly supplied through the narrow channels that are being increasingly observed and seem to be directed from the compact cores. Continuous production of high-energy plasma in a high-pressure region at the core centre is necessary to account for the energetics and channel structures¹. The twin-exhaust mechanism² is one means of forming double jets in active galactic nuclei. As originally envisaged², the 'central engine' continuously releases hot gas in the centre of a flat-bottomed potential well formed by the stellar cluster in a galactic centre. This potential well holds a cloud of cooler gas which confines the hot gas in a central cavity. If the cooler gas cloud is flattened (for example, by rotation), buoyancy forces can accelerate the hot gas into two collimated jets via de Laval nozzles. These jets may correspond to the observed radio jets in quasars and radio galaxies, and result in the formation of extended double sources. Here, we combine new results from numerical calculations³ of the Blandford-Rees model², with severe observational and theoretical constraints, to reach conclusions concerning the nature of the allowed flows. In particular, we conclude that high-velocity, high-powered jets cannot be produced in gravitational potentials produced by stellar clusters. However, the more general notion of the twin-exhaust mechanism¹, in which the central potential can be cusp-like near a massive black hole, is not ruled out for the high-powered radio sources. Note also that more complex twin-exhaust models (for example, magnetohydrodynamic flows) may lead to less severe constraints.

Blandford and Rees² obtained analytical results by treating the cavity flow as that of a one-dimensional relativistic fluid with the containing cloud supported by thermal pressure in a flat-bottomed potential well. They proposed central clouds of size ≤ 100 pc. Wiita³ demonstrated that a cloud as small as 1 pc is still consistent with X-ray observations. Furthermore, he also used a '1½' dimensional numerical method⁴ to study the formation process and discovered a lower limit to the power for which jets form. Recently, a fully two-dimensional axisymmetric code has been used⁵ to explore the formation process, with full inclusion of nonlinear hydrodynamic instabilities. Several interesting results were obtained and are summarized below.

A self-gravitating cloud with an axial pressure ratio of 2:1 was used and non-relativistic hot gas introduced with specific energy ~ 10 times greater than the cloud's specific energy, ϵ . For a certain central luminosity range ($L_{\min}$, $L_{\max}$), nozzles do form⁵, but are more compact than those assumed previously^{2,4}. Above $L_{\max} \sim 2.0\epsilon^{5/2}G^{-1}$, the jet collapses and large clouds are ejected. Below $L_{\min} \sim 0.05\epsilon^{5/2}G^{-1}$, the jet continuously pinches off and reforms and small bubbles rise out along the symmetry axis. These limits are also seen to be limits on the nozzle radius r_n —jets form when $0.1h < r_n < 0.6h$, where h is the pressure scale height in the cloud. These results were analytically generalized by careful consideration of the cavity structure and instabilities (for details see ref. 6). The lower limit is due to the Kelvin-Helmholtz instability breaking up thin nozzles, whereas the upper limit is due to the Rayleigh-Taylor instability along the cavity walls. Thus we found that jets form between

$$\frac{L_{\max}}{10^{46} \text{ erg s}^{-1}} = 2.0\xi \left(\frac{c_1}{3c_0} \right) \left(\frac{10^7 M_g}{M_t} \right) \left(\frac{c_0}{10^4 \text{ km s}^{-1}} \right)^5 \quad (1)$$

and

$$\frac{L_{\min}}{10^{46} \text{ erg s}^{-1}} = 0.07\xi \left(\frac{c_1}{3c_0} \right)^{7/3} \left(\frac{10^7 M_g}{M_t} \right) \left(\frac{c_0}{10^4 \text{ km s}^{-1}} \right)^5 \quad (2)$$

where c_0 and c_1 are the sound speeds in the cloud and hot gas, respectively; M_g and M_t are the mass of gas and total mass, respectively, within h ; and $\xi c_0^2 = GM/h$, that is,

$$\frac{h}{1 \text{ pc}} = 0.7\xi^{-1} \left(\frac{M_t}{10^{10} M_\odot} \right) \left(\frac{c_0}{10^4 \text{ km s}^{-1}} \right)^{-2}. \quad (3)$$

These can be derived from $L \propto \rho_1 c_1^3 r_n^2$, where ρ_1 is the jet density⁶. The maximum follows directly from the condition $r_n < 0.6h$. The effectiveness of the Kelvin-Helmholtz instability results in the higher exponent of $(c_1/3c_0)$ in the minimum jet power (this extra exponent of 4/3 is a combination of jet thrust (2) and growth rate and phase velocity $(-2/3)$). It follows that jets cannot be maintained for $c_1 > 30c_0$.

We note the critical dependence on c_0 . If c_0 typically varies between 10^3 km s^{-1} and 3.10^4 km s^{-1} , then the jet power can

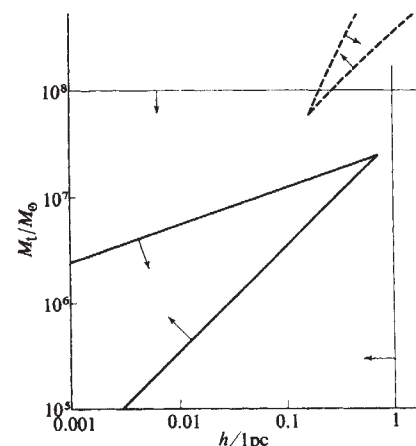


Fig. 1 The constraints on galactic nuclei mass M_t and scale height h for jets of power $10^{46} \text{ erg s}^{-1}$ in the optically thick case as described in the text. Both observational (solid lines) and theoretical constraints (broken lines) cannot be simultaneously satisfied.

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range between 10^{41} erg s $^{-1}$ and 10^{48} erg s $^{-1}$. Such a wide range is necessary to cover all sources with jets or extended components. We suggest that these formulae will also hold for a radiatively supported cloud, taking $c_0^2 \sim p_r/\rho$ where p_r is radiation pressure and ρ is the mass density, and also for a relativistic gas after adjustments are made for relativistic effects that will involve factors of $1 - c_1^2/c^2$. Without the support of numerical simulations and given the complexity of three-dimensional fluid flows, these extensions are tentative.

We list below the observational constraints imposed by quasars and the compact central components of radio galaxies.

(1) $h < 1$ pc. This comes directly from very long baseline interferometry observations (there is also a class of radio galaxies with sizes of a few kpc that will not be considered here)⁷.

(2) Collimated energy outputs, L_j , between 10^{41} erg s $^{-1}$ and 10^{46} erg s $^{-1}$ are typical over long time scales. The quasar jet in 4C 32.69 may require up to 10^{48} erg s $^{-1}$ (ref. 8). Directed outbursts in strongly varying radio quasars often require 10^{48} erg s $^{-1}$ over periods of a few years⁹. The upper end of the jet power range provides the severest constraint when combined with equation (1).

(3) X-ray luminosities from quasars are in the range $L_x \sim 10^{43}$ – 10^{47} erg s $^{-1}$ (refs 10, 11). Only a fraction of this is thought to occur by thermal emission. Thus, although less extreme cases exist, we can place a severe limitation on quasars with jets by taking $L_{\pi} \leq 0.1 L_j$, where L_{π} is the free-free emission from the cloud. This constraint will then also apply to radio galaxies.

To these we add the following theoretical constraints.

(4) $\rho_s = 10^9 M_{\odot} (1 \text{ pc}/h)^{2/3} \text{ pc}^{-3}$ is a maximum stellar density for ordinary stars^{12,13}, derived by requiring a star collision time $> 10^8$ yr. Above this value, star mergers occur and the star cluster is rapidly converted into a supermassive star. This value is extremely high and we believe that the maximum ρ_s may in fact be smaller. Greater densities are possible if collapsed stars are present.

(5) Mass outflows from galactic nuclei $> \dot{M} \sim 1,000 M_{\odot} \text{ yr}^{-1}$ lead to enormous difficulties¹. There is no known mechanism for continuously supplying the central engine at such a rate over long time scales. As $L_j = 24 c_0^2 \dot{M}$ (with $c_1 = 3c_0$), limits on $\dot{M}$ force limits on L_j .

Constraints (1) and (2) can be satisfied for a portion of the parameter space (M_g, M_t), given c_0, c_1 and ξ . It is convenient to rewrite constraint (1), using equation (3) as ($\xi = 1, c_1 = 3c_0$):

$$\frac{M_t}{10^{10} M_{\odot}} < 1.4 \left(\frac{c_0}{10^4 \text{ km s}^{-1}} \right)^2, \quad (4)$$

and to bound M_g by constraint (2) using equations (1) and (2):

$$\frac{M_g}{10^4 M_{\odot}} = (0.05, 1.43) \left(\frac{M_t}{10^{10} M_{\odot}} \right) \left(\frac{c_0}{10^4 \text{ km s}^{-1}} \right)^{-5} \left(\frac{L_j}{10^{46} \text{ erg s}^{-1}} \right) \quad (5)$$

Near the limiting values, much of the flow is disrupted by instabilities, perhaps giving rise to the observed radio jets. Efficient energy transfer will occur in the range $\sim (0.1, 0.7)$ of the bracketed quantity in equation (5), and we shall take the mean to be 0.4. We have also taken $c_1 = 3c_0$. Because $L_{\min} \rightarrow L_{\max}$ as $c_1 \rightarrow 30c_0$ this will not greatly affect our results.

First consider the case where the confining cloud is optically thin, that is, the optical depth, τ , of the cloud is less than unity. The thermal X-ray emission is given by³

$$\frac{L_{\pi}}{L_j} \sim 3 \cdot 10^{-2} \left(\frac{M_g}{10^4 M_{\odot}} \right) \left(\frac{10^{10} M_{\odot}}{M_t} \right)^2 \left(\frac{c_0}{10^4 \text{ km s}^{-1}} \right)^2 \times \left\{ 1 + 2.7 \left(\frac{c_0}{10^4 \text{ km s}^{-1}} \right)^2 \right\} \leq 0.1. \quad (6)$$

This constraint is stronger than the optical depth condition. When equations (4), (5) and (6) are combined, a lower limit to c_0 is obtained:

$$\frac{c_0}{10^4 \text{ km s}^{-1}} > 0.6 \left\{ 1 + 2.7 \left(\frac{c_0}{10^4 \text{ km s}^{-1}} \right)^2 \right\}^{1/5} \left(\frac{L_j}{10^{46} \text{ erg s}^{-1}} \right)^{1/5} \quad (7)$$

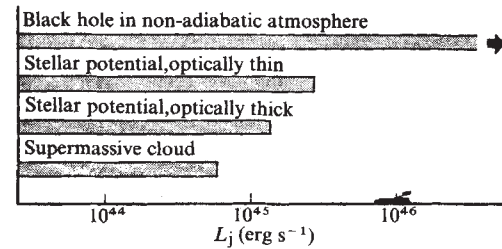


Fig. 2 The allowed luminosity range for various possible confining clouds.

An upper limit for c_0 is obtained by invoking constraint (4) and eliminating h using equation (3): $(c_0/10^4 \text{ km s}^{-1}) < 0.7 (M_t/10^{10} M_{\odot})^{2/7}$. This yields the result that if we wish the flat-bottomed potential to be produced by stars, then $c_0 < 5.3 \times 10^3 \text{ km s}^{-1}$. That is, the twin-exhaust model for a stellar cluster necessitates low sound speeds. Due to the restriction $c_1 < 30c_0$, stellar cluster models cannot produce the stable relativistic jets previously assumed^{2,3}. Using equation (7), the maximum jet power is 2.7×10^{45} erg s $^{-1}$. These limits to c_0 and L_j may be related to head-tail radio galaxies and other weak radio sources, where low jet speeds have been inferred^{14,15}.

We can find further solutions involving stellar potentials if the cloud is optically thick. Here $c_0^2 \sim p_r/\rho \sim GM_t/h$ where p_r is the radiation pressure and ρ is the mass density. The median jet luminosity can be rewritten as ($c_1 = 3c_0, \xi = 1$):

$$\frac{L_j}{10^{46} \text{ erg s}^{-1}} \sim 1.6 \left(\frac{M_g}{10^7 M_{\odot}} \right) \left(\frac{M_t}{10^8 M_{\odot}} \right)^{3/2} \left(\frac{1 \text{ pc}}{h} \right)^{5/2}. \quad (8)$$

The total radiation limits M_t through the Eddington limit. Here we take

$$\frac{M_t}{10^8 M_{\odot}} = \frac{L_{\text{edd}}}{10^{46} \text{ erg s}^{-1}} < \frac{L_j}{10^{46} \text{ erg s}^{-1}}. \quad (9)$$

This proves to be unimportant in the following. Further constraints are $M_g \leq M_t$, and $M_g/10^7 M_{\odot} > 4.3 (h/1 \text{ pc})^2$ is the condition for the optical thickness of the jet to exceed 10 (if the cloud is optically thick, but the jet is optically thin, the advantages of taking an optically thick cloud are lost). For $M_g < M_t$, the optically thick and observational constraints (1–3) can be satisfied (Fig. 1) only if $M_t < 3 \times 10^7 M_{\odot}$ for the twin-exhaust model. The theoretical constraints (4, 5), however, require $M_t > 8.4 \times 10^7 M_{\odot}$ for $L_j = 10^{46}$ erg s $^{-1}$. Thus we find two possibilities: either $L_j < 1.4 \times 10^{45}$ erg s $^{-1}$ or the mass is not in the form of ordinary stars. The second possibility necessitates masses of an order of magnitude above that given by constraint (4). Thus, again we have found that stellar cluster models are implausible for high powered jets.

Finally, we can look at supermassive clouds by taking $M_g = M_t$ and so dropping constraint (4). Use of constraint (5) in equation (8) yields $L_j < 6 \times 10^{44}$ erg s $^{-1}$, after eliminating h using equation (3). Here, the low sound speed of the dense cloud is prohibiting powerful jets.

There are two alternatives to assuming that M_t is in the form of stars. The first is to have the mass in a cluster of collapsed objects. With the increased collision times, constraint (4) can be relaxed, but we will not pursue this possibility here. The other is that the mass is primarily in a central black hole that will produce a cusp-like potential ($1/r$). Such supermassive objects can meet the mass requirements of either of the above models and are consistent with observations¹⁶.

Can jets form in steep potential wells? The pressure distribution of a stationary adiabatic atmosphere surrounding a point mass is of the form $Par^{-(\gamma/(\gamma-1))} ar^{-5/2}$ for a specific heat ratio $\gamma = 5/3$ where r is the radial distance. It was found⁶ that the cavity structures analysed here will only be produced for Par^{-m} with $m < 2$. Thus adiabatic atmospheres are not permissible. A radiatively cooling cloud will have a flatter pressure distribution. With pressure dominating gravity, the mass requirements are eased; moreover, the rate of cooling leads to a central accretion

rate consistent with the mass ejected through the jets⁶. We further suggest that the cloud sound speed is $\sim 10^4 \text{ km s}^{-1}$, as then the jet velocity will be nonrelativistic, $\sim 0.1c$, which agrees with expectations^{1,17}. This model predicts that jets approaching relativistic speeds, that is, $c_1 \rightarrow 30c_0 \sim L_{\min} \rightarrow L_{\max}$, will be highly variable and short-lived, perhaps giving rise to the rapidly separating discrete components observed in such sources, and intrinsic outbursts with $\geq 10^{47} \text{ erg s}^{-1}$.

We conclude that the twin-exhaust model for radio jets cannot be ruled out, but that distinct classes of confining clouds (Fig. 2) may occur. The low-powered jets ($L_j \leq 10^{45} \text{ erg s}^{-1}$) can be maintained by a flat-bottomed stellar cluster potential. These jets must have nonrelativistic jet velocities, which could be as low as $10^2\text{--}10^3 \text{ km s}^{-1}$, comparable with the interstellar velocities which the jets may encounter on the way out of the 'central engine'. Thus, these low-power jets could still have black hole sources ($r \sim 10^{-5} \text{ pc}$), but with the jet flow becoming randomized before being refocused by de Laval nozzles on scales of $\sim 1 \text{ pc}$ where the gravitational potential is dominated by the stellar cluster. It seems that if the stars are replaced by a central black hole with a cusp potential and a non-adiabatic atmosphere, then powerful jets are attainable. It is hoped that the above analysis will suggest future observations which will distinguish between these models.

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Glass formation in ionic systems

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In the past five years, glass formation has been observed in many chemical systems involving zirconium tetrafluoride¹⁻⁵. These ionic glasses were unexpected as no theory of glass formation had predicted their occurrence. Most common glasses can be explained by the Zachariasen theory⁶ which gives criteria for vitreous structures with highly directed three-dimensional bond arrays, according to energy and kinetic considerations. However, the theory does not encompass some new amorphous systems such as amorphous germanium or the new ionic glasses. I review here the structural and energetic conditions for glass formation in ionic systems with particular reference to fluoride glasses.

In some non-stoichiometric compounds, especially in 'anion-excess' phases, the structure includes both order and disorder elements, the cations describing a regular network related to the

average crystallographic cell and the anions being situated in a disordered way over equivalent positions around the cationic sites. Examples are UO_{2+x} (ref. 7), $(\text{CaY})\text{F}_{2+x}$ (ref. 8), MF_{2+x} (ref. 9) and $(\text{Zr, Ln})(\text{O, F})_{3+x}$ (ref. 10). Which all have cubic symmetry. A cubic distribution maximizes the cation-cation distances and therefore minimizes their coulombic repulsion. If this regular cubic distribution may be precluded, for example, by addition of larger cations, then the cationic array may also be disordered. Such a relationship exists between the non-stoichiometric phases $(\text{Ln, Zr})\text{F}_{3+x}$ (ref. 11) and the ternary $\text{ZrF}_4\text{--LnF}_3\text{--BaF}_2$ glasses (ref. 12).

The predominant role of the anions in disordered ionic solid state phases enables the problem of glass formation to be addressed by considering the additional features leading to stabilization of a non-periodic packing. This approach involves both energetic and ordering elements. Glass formation is allowed if the rate of nucleus formation and the velocity of crystal growth are low enough to avoid noticeable recrystallization of the melt between liquidus and glassy transition. This may be achieved when the activation enthalpy for shear viscosity is high. In the case of covalent network glasses, the structural implications are expressed by Zachariasen rules. For ionic glasses, the first obvious condition is that the mobility of highly charged cations in the structure is low. Besides, constructing a non-periodic structure implies that there are several ways in which the cations can be inserted in the anionic packing, which is also correlated to the fact that the energy of the glassy structure must be close to the energy of the crystalline forms that derive from it.

The lattice energy of the solid W_s may be written as: $W_s = W_E + W_R$ where W_E is the electrostatic energy and W_R the repulsion energy resulting from the overlapping of the electronic shells. The electrostatic term W_E may be rigorously calculated by summation of convergent series in direct or reciprocal space if the precise structure is known¹³. It may be expressed as the sum of the repulsive anionic energy W_{RA} , the repulsive cationic energy W_{RC} , and the interaction energy between ions of opposite sign W_I .

$$W_E = W_I + W_{RA} + W_{RC}$$

with $W_I < 0$, W_{RA} and $W_{RC} > 0$.

The predominant part is W_I which is proportional to the product of the cationic charge n and the anionic charge z . W_{RA} is proportional to z^2 and W_{RC} to n^2 . W_E will increase with increasing n , and decreasing bond lengths, as long as the repulsive part W_{RC} is not too important. In a vitreous ionic structure, only a short-range order exists and therefore the coulombic energy restricted to near-neighbour ions is likely to be similar for crystals and glasses. The medium- and long-range coulombic

Table 1 Glass forming ability of various cations with respect to anionic and cationic field strength

Cation	Anion	F_A^*	F_C^*	F_A/F_C	Glass progenitor	Ref.
Al^{3+}	F	0.78	5.6	7.2	Yes	19
Be^{2+}	F	0.78	7.4	9.5	Yes	19
Zr^{4+}	F	0.78	5	6.4	Yes	12
Hf^{4+}	F	0.78	5.2	6.7	Yes	20
Ga^{3+}	F	0.78	4.8	6.1	Yes	14
Fe^{3+}	F	0.78	4.7	6.0	Yes	14
Cr^{3+}	F	0.78	4.9	6.3	Yes	14
Th^{4+}	F	0.78	3.8	4.9	Yes	15, 16
Y^{3+}	F	0.78	3.1	4.0	Yes	21, 22
Zn^{2+}	F	0.78	2.8	3.6	Yes	22
Si^{4+}	F	0.78	15.4	19.7	No	
Mo^{6+}	F	0.78	14.6	18.7	No	
Ca^{2+}	F	0.78	1.78	2.3	No	
NaF	F	0.78	0.85	1.1	No	
Zn^{2+}	Cl	0.55	3.33	6.0	Yes	19
Bi^{3+}	Cl	0.55	2.91	5.3	Yes	23

* Calculated from the ionic radius values given in ref. 24.

contributions may, however, differ significantly. A maximum interaction energy is reached when the set of the anion-cation distances is minimum and hence the anion packing is most compact.

Glass formation is most likely for an ionic material if the relative part of the short-range energetic terms is important and there is a high potential barrier between the cationic sites as implied by a high cationic site potential. Thus, the first condition for a disordered distribution of the cation is that the anion packing must exhibit many more possible sites than actually needed. This is favoured by a large anion/cation ratio, and depends also on the size and the geometry of the sites. For example, in a compact packing of spheres, there are two tetrahedral sites and one octahedral site per anion. Small sites are generally more numerous, and the cation which can possess several different coordination numbers has also more insertion possibilities. The second condition relates to the packing itself which must not be close-packed because otherwise ordered structures such as close-packed hexagonal or face centred cubic would appear. Finally, the ordering forces arising from the cationic repulsion must be minimized.

The different elements to be taken into account dealing either with electrostatic energy (E) or with ordering (O) criteria are summarized below:

E_1 is the interaction energy between anions and cations.

E_2 is the intercationic repulsive energy.

E_3 is the repulsive energy of the anion packing.

O_1 is the ratio (number of empty sites)/(number of cations).

O_2 Avoids a close-packed arrangement of anions.

O_3 Minimizes ordering forces induced by cationic repulsion.

In view of these elements, the cations exhibiting a high field strength n/R appear as potential vitrifiers as they induce a high stabilization energy W_1 and a large anion/cation ratio. Moreover, they are usually small, which increases the number of potentially available sites. It seems that glass formation in ionic systems cannot normally be observed when all the cations exhibit a field strength lower than a limiting value which depends on the average anionic field strength F_A .

On the other hand, when the cationic field strength is high compared with the anionic field strength, then the repulsive cationic energy W_{RC} is large in comparison with the interaction energy W_1 , so that the ionic character of the bonding disappears and some competition occurs between a tridimensional—and therefore solid state—structure and molecular arrangements, resulting usually in gaseous or liquid phases. In practice, the criterion $10 > F_C/F_A > 2.5$ could be used to predict whether a cation may be a glass progenitor. In the usual case of multi-component glasses, the strength F must be weighted by the atomic fraction x_i , so that the factor $P_i = x_i F_i$ evaluates the contribution to the vitrification.

Larger cations may enhance the likelihood of glass formation both by influencing the kinetics of the crystallization process and by decreasing the average intercationic repulsion (E_2) and therefore the ordering forces (O_3), and also the anionic repulsion in the dislocation areas (E_3). Moreover, the introduction of large cations is a good way of precluding a close-packed anion array, as they do not usually insert in tetrahedral or octahedral sites (O_2). Such cations exhibiting a weak field strength are usually called 'modifiers' in the sense that they 'modify' the periodicity of the network, here this term describes the modification of the compactness and the electric field.

The zirconium fluoride glasses are the best example of ionic glasses. The Zr^{4+} ion exhibits a medium field strength ($Z/R = 5$), but it may insert in in various sites with coordination number of 6, 7 or 8. Its advantage is that it will stabilize a fluoride packing in which a high anion/cation ratio will pertain and the repulsion arising from its rather high charge ($4+$) may be compensated both by large cations with lower field strength (Ba^{2+} , La^{3+} ...) and by F-F bridges (ref. 10).

The glass-forming ability of various cations is reviewed in Table 1 according to the anionic and cationic field strengths. In

fluoride systems, many new glasses have recently been prepared from trifluorides and tetrafluorides associated with modifiers and sometimes with transition metal difluorides¹⁴⁻¹⁶. Note that lithium may insert in both tetrahedral and octahedral sites: LiF-rich glasses have thus been synthesized either in association with metaphosphates which decrease the average anionic field strength¹⁷, or combined with a few per cent of tetrafluoride, which increases both the anion/cation ratio and the average cationic field strength¹⁵.

The BeF_2 and $ZnCl_2$ glasses¹⁸ may also be described as ionic glasses, rather than by analogy with covalent glassy SiO_2 . Fluorophosphate glasses in some ranges of composition¹⁷ cannot be considered as continuous network glasses and therefore an ionic analysis would be more correct. Finally, ionic salt glasses—carbonates, nitrates, hydrogenosulphates¹⁷—are also relevant to this analysis with the specific aspect of the non-sphericity of the NO_3^- and CO_3^{2-} ions.

Clearly the concept of a vitreous network involving covalently directed bonding does not apply to ionic fluoride glasses. The present discussion provides a framework for understanding the existence of the fluoride glasses and provides guidance for predicting new ionic glass-forming systems. These ionic glasses will prove important in new technological fields such as optical fibres and IR transmittance¹².

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Do graded units of accretionary spheroids in the Barberton Greenstone Belt indicate Archaean deep water environment?

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We report here a re-analysis of the sedimentology of the Msauli Chert ('oolite') which shows that environmental conditions during the early depositional phase of the Fig Tree Group in the Barberton Greenstone Belt were of a deep water nature. The unit is compared with Quaternary sediments deposited in the Pacific Ocean at bathyal to abyssal depths.

The Msauli Chert is a pale green-grey chert unit about 20 m thick in which the silica is clearly of a secondary nature. In

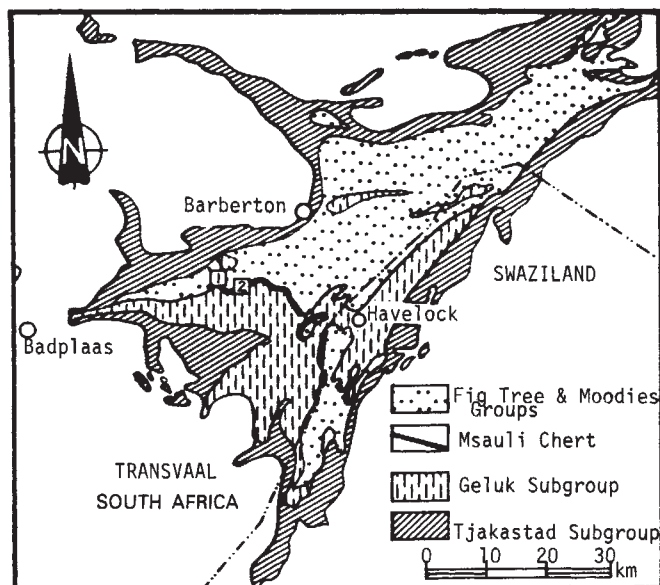


Fig. 1 Generalized geological map of the Barberton Greenstone Belt with the Msauli Chert localities marked.

stratigraphical terms the unit has been placed either at the base of the largely sedimentary Fig Tree Group¹ or near the top of the Swartkoppie Formation², the uppermost formation of the underlying $3.3\text{--}3.5 \times 10^6$ Myr old^{3,4} Onverwacht Group (Figs 1, 2) which underlies the Fig Tree Group. The latter largely comprises extrusive and intrusive mafic and ultramafic rocks.

The Msauli Chert is characterized by interspersed 2 cm and 30 cm thick units of concentrically laminated spheroid grains up to 10 mm in diameter (designated accretionary spheroids). Whereas most descriptions of this lithology from the Barberton Greenstone Belt^{1,2,5-7}, agree on the accretionary nature of these spheroids, the formation process is controversial. Interpretations of the accretionary spheroids include: (1) shallow water carbonate¹ ooids deposited as widespread oolite shoals analogous to those described from the Bahamas Platform^{8,9}; (2) accretionary lapilli¹⁰ (found in both recent and ancient pyroclastic deposits within subaerial volcanic terrains¹¹⁻¹⁷) which fell into a shallow water body; and (3) pisoliths (M. J. de Wit, R. Hart, A. Martin and P. Abbott, in preparation) formed near recent subaerial hydrothermal vents, such as those associated with geysers of the Yellowstone Park¹⁸ and Taupo geothermal zone, New Zealand (M. J. de Wit *et al.*, in preparation). These possibilities suggest a shallow marine or subaerial origin for the grains, and this has been used to substantiate a shallow water environment for the Msauli Chert^{9,10}. In contrast, other studies of the Fig Tree Group in the southern part of the Barberton Greenstone Belt have emphasized its deep water nature¹⁹. In addition, the Msauli Chert is associated with volcanic rock sequences that have been directly compared with oceanic crust^{20,21}. In these sequences pillow lavas are predominantly non-vesicular and have abundant variolitic structures², suggesting a deep water environment ($>1,500$ m) by analogy with studies of modern oceans²². This conflicting evidence has prompted us to re-examine the Msauli Chert and assess the environment it represents by studying other sedimentary features associated with the unit.

During a regional geological mapping programme in the southern part of the Barberton Greenstone Belt (M.J.d.W., 1978-81), two excellent exposures (Fig. 1) of the Msauli Chert were discovered on the farms Clarendon 714JT and Granville Grove 720JT. The first locality enables the unit to be studied in the laboratory and the second exposes a water-polished section suitable for detailed sedimentological analysis of the unit (Fig. 3).

At the second locality the Msauli Chert comprises normally graded units 2-85 cm thick (Figs 3, 4a). These units have sharp bases which are often erosional. The spheroids commonly comprise the coarse basal part of the graded units (Figs 3 and 4a,

b), are usually <3 mm and rarely up to 10 mm in diameter. Their cortex is concentrically laminated and may have a definite nucleus, sometimes deep green in colour. The spheroids are predominantly microquartz chert ($<30 \mu\text{m}$) with interspersed sericite. The nuclei vary from angular to rounded and contain a high proportion of sericite mica. The space between spheroids is filled with chert grading from microquartz to megaquartz, which in parts follows a pre-existing radiating fibrous chalcedony.

The top of each normally graded unit comprises grains of silicified sand- to silt-grade material with thin beds of black chert that may represent silicified carbonaceous mudstone in places (Fig. 3). However, black chert layers may have various origins²⁴ and may even form as veins. The silicified sand- to silt-grade material shows well preserved sedimentary structures (Fig. 4b), including planar lamination, ripple and climbing ripple cross-lamination (types A and B and sinusoidal type²⁵), mudstone-siltstone interlamination, convolute lamination (Fig. 4c) and very rare dune-form cross-bedding (Fig. 3). Accretionary spheroids are occasionally included in planar laminated, cross-laminated and cross-bedded units.

Most of the graded units show ordered sedimentary structures like that of turbidites^{26,27} (Figs 3 and 4a, b). Either the top of the Bouma sequence²⁶ is missing (Fig. 4b) because of the erosive base of the succeeding graded unit (that is, they are generally amalgamated) or the bottom part of the Bouma sequence is missing, probably due to the variation in the initial flow velocities of the depositing current or to the grain size distribution of the sediment available for redeposition. 72% of the units fall into this classic turbidite category (ABC, BC, AB, ABCD and so on), 14% show only one Bouma division (A, B, C) and another 14% have one division missing (AC, BD, ABD).

This resemblance to Bouma sequences has been reported previously¹⁰, although it was noted that the A-division only rarely passed upwards into a B division. From the polished exposure described here it can be shown that of graded units starting with an A division, 80% pass upwards into a B division and only 20% pass upwards into a C division. Previously reported is a high degree of sorting in the basal few centimetres of a particular A division¹⁰ and similarly in this study of the graded units investigated, one unit from each locality shows a layer at the base of the A division that is far better sorted than the rest of the division. These layers are 5 cm (Fig. 4b) and 7 cm thick respectively, and consist of large accretionary spheroids 1-10 mm in diameter, with the large spheroids scattered randomly within the small spheroids (there is no grading). The layers are devoid of the finer interstitial sand and silt material that characterizes the remainder of the spheroid-rich A division. The poorly sorted graded upper part of the A division was probably deposited in the turbulent conditions of a classic high-velocity turbidity current and the grains which comprise the thin basal

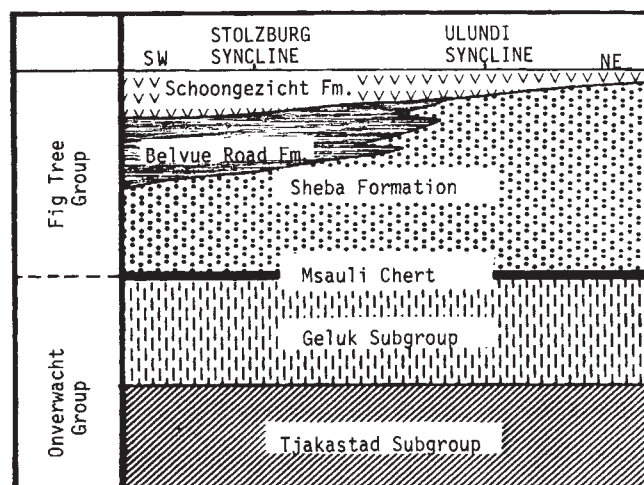


Fig. 2 Generalized stratigraphy of the Onverwacht and Fig Tree Groups (modified from refs 1, 45).

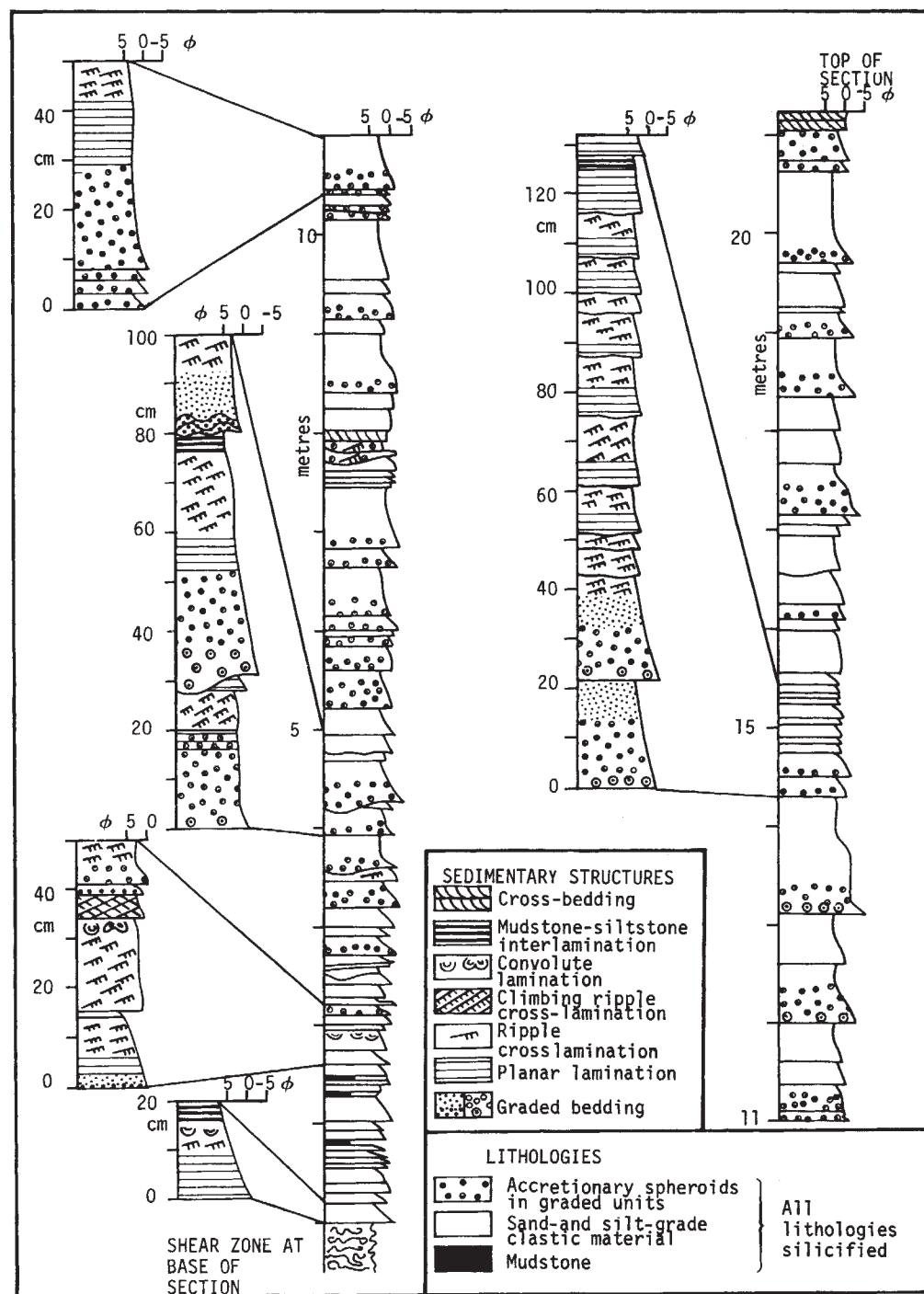


Fig. 3 Detailed measured section through the Msauli Chert at locality 2. Grain size variation is plotted in ϕ units.

layers described were probably transported under the influence of grain dispersive pressure, as true grain flows²⁸. It is possible that the turbidity currents in these two cases were riding on a carpet of cohesionless grains experiencing the Bagnold effect²⁹, and that the basal layer was preserved by the 'freezing' of this carpet, perhaps because of a change in depositional slope. The upper part of the unit was then deposited under the influence of the turbidity current. The basal layers described above approximate to the calculated theoretical maximum thickness of 5 cm for grain flow deposits³⁰, thus supporting this interpretation. The grain flow layer from locality 2 occurs at the base of one of the thicker (68 cm), more proximal graded units, where such a layer might be expected to develop.

Of the three previous environmental interpretations for the depositions of the Msauli Chert listed above, the oolite shoal hypothesis is the least tenable. Studies of both recent and ancient oolite shoal bodies of the Bahamian type emphasize the development of large-scale cross-bed sets 0.4–4 m thick^{8,31}.

Structures as big as these are not present at the localities studied. It would also be difficult to explain the development of so many graded units in terms of a shallow carbonate model. However, it is possible for carbonate grains such as ooids to be transported by storm or tidal currents over the edge of a carbonate shelf or platform, to be deposited at the base of the slope. Limestones deposited in such a way have been described from the ancient^{32–36} and Quaternary examples have also been described^{37,38}. The deposition of the graded units at the base of a shelf or platform slope would be consistent with the identification of grain flow bases to the units, because grain flows require considerable slopes to maintain themselves^{28,30,39}.

The interpretation of the grains as accretionary lapilli¹⁰ implies an association with an air-fall volcanic ash. These were considered to have fallen into shallow water to allow the separation of lapilli from sand- and silt-grade ash material, to develop graded units by differential settling. To explain the sedimentary structures described from the tops of the graded

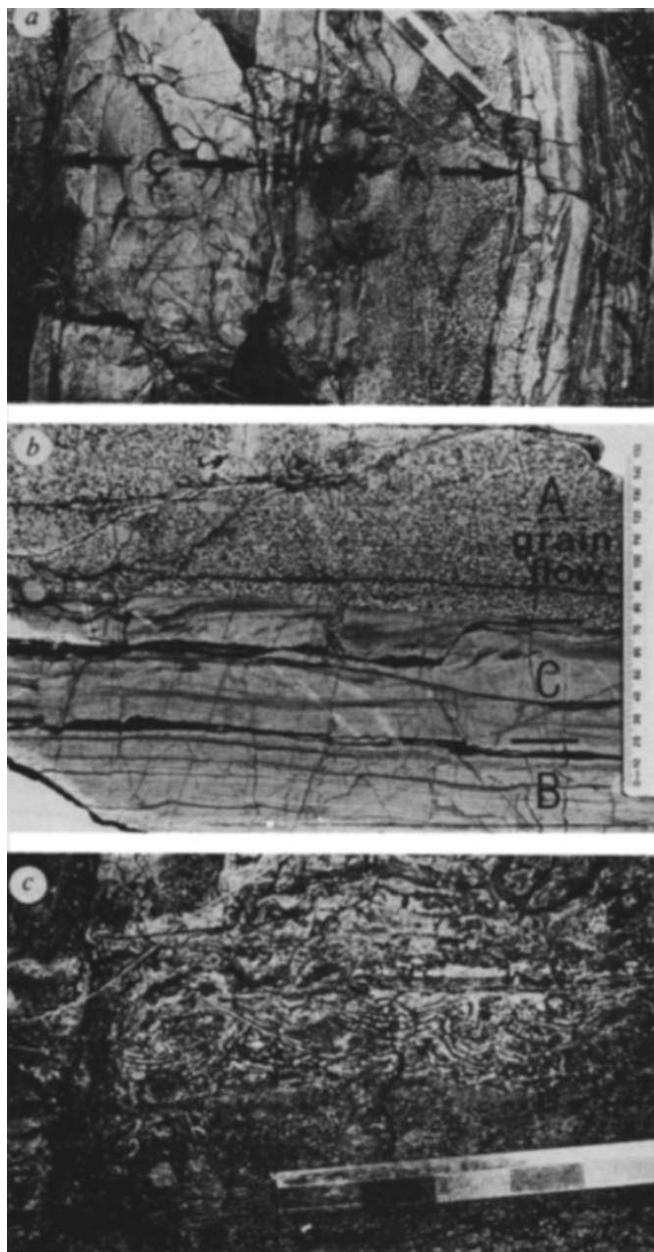


Fig. 4 Sedimentary features in the Msauli Chert indicating deposition by turbidity currents: *a*, a graded unit with postulated Bouma divisions marked. Subdivisions on the scale are 5 cm long; *b*, sawn section through the grain flow base of a graded unit overlying planar laminated and ripple cross-laminated sand- and silt-grade material of the underlying graded unit. Postulated Bouma divisions are marked; *c*, convolute lamination at the top of a graded unit. Subdivisions on the ruler are 5 cm long.

units, 'tidal currents' were invoked to rework the top of each graded unit. Although the evidence presented here does not rule out the possibility that the grains were accretionary lapilli, this mode of formation for the graded units is unlikely because: (1) the tidal currents would have to rework unreasonably large volumes (50–90%) of each unit; (2) it is doubtful whether tidal currents would produce such a high ordering of sedimentary structures; (3) the interpretation does not predict the development of erosional bases to the graded units.

The possible interpretation of the spheroids as accretionary lapilli complicates the interpretation of an environment of deposition for the graded units. As well as forming in airborne ash clouds, accretionary lapilli may form in base surges¹³—pyroclastic-laden currents which spread out from the base of a vertically rising volcanic ejecta column. These have been classified as a type of density current¹⁵. They can generate

subaerially a normally graded unit containing sedimentary structures. However, work on Recent base surges^{13–17,40–43} indicates that they resemble turbidity currents in process but not in product. Although base surge deposits can be normally graded, they are often reversely graded^{14,16,40}. Also the preservation of upper and transitional flow regime sedimentary structures such as antidunes and plane bedding^{14,15,40} is far more common than in turbidity current deposits. Where lower flow regime structures are documented from base surge deposits, large-scale dune-form cross-bedded sets 20–100 cm thick^{14,15,40} are described, rather than the ripple cross-lamination reported here. If accretionary lapilli are preserved within a base surge deposit, they tend to concentrate near the middle or top of that deposit¹⁴. Clearly the graded units described from the Msauli Chert cannot be ascribed to the deposition of subaerial base surges. However, a base surge that enters a water body such as a lake or open sea might transform into a classic turbidity current and be redeposited in a similar fashion to the graded units described here. In fact, such a depositional process has been described from a volcanic lake¹⁶. Whether accretionary lapilli would survive such a transformation from base surge to turbidity current has not been reported and remains doubtful until proved.

The graded units from the Msauli Chert described here are interpreted as the product of turbidity currents *sensu stricto*, in which grain flow deposits are rarely preserved as thin basal layers. We therefore maintain that although the accretionary spheroids originated in shallow water or subaerial environments, they were later redeposited at considerable depth. This agrees with evidence from underlying rock types, non-vesicular, variolitic, mafic pillow lavas which indicate considerable oceanic depths.

Closely analogous to these ancient sedimentary deposits are the graded units of greenish-grey sediment, 5–80 cm thick, which are found in the equatorial Pacific Ocean³⁷. They consist of a mixture of shallow water carbonate grains derived from the nearby Line Islands mixed with volcanoclastic grains and deep water oozes, and are thought to have been deposited by a variety of gravity flow types including turbidity currents, which transported sediment derived from shallow marine carbonate complexes, shallow and/or deeper water volcanic terrains and basinal deep marine environments into oceanic water depths of ~4,500 m. Distances covered by the depositing gravity flows would be of the order of tens to hundreds of kilometres.

Although these conclusions suggest that the Msauli Chert was deposited at oceanic depths, the latter were probably far less during the Archaean than at present⁴⁴, perhaps as little as 2,000 m.

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Shallow water and hypersaline features from the Middle Proterozoic Mt Isa Sequence

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Fundamental differences of opinion exist about the depositional environment of sediments which host the Middle Proterozoic¹ Mt Isa Cu and Pb–Zn deposits^{2,3} of northwestern Queensland, Australia. The copper host has been repeatedly interpreted as an algal reef^{2,4}, and recently as a sabkha⁵, whilst models as diverse as saline lakes⁶ and deep oceanic basins^{3,7–9} have been proposed for the deposition of both the Cu and Pb–Zn orebodies. We now report the discovery of stromatolites, halite casts, large cross-bedded channel deposits, and flat-pebble conglomerates in sediments closely associated with the ore deposit. These sedimentary structures provide evidence of shallow-water deposition with intermittent hypersaline and emergent conditions during deposition of the Upper Mt Isa Group^{3,6} sequence. The assemblage of sedimentary structures strongly supports a shallow-marine or shallow-lacustrine depositional model. Either model is inconsistent with the previously postulated Red Sea-type submarine exhalative mineralization model^{3,7–9} for Mt Isa. Instead we argue that Mt Isa ore formation took place in a shallow-water system, possibly aided by the action of evaporite-derived brines.

The Mt Isa Cu–Pb–Zn deposit, one of the world's largest accumulations of metal sulphides, consists of stratiform Pb–Zn ores alongside major Cu orebodies. The Cu mineralization (chalcopyrite, pyrite, pyrrhotite) is hosted by a discordant and brecciated dolomite–quartz mass, whereas Pb–Zn ores (galena, sphalerite, pyrrhotite) are interbedded with dolomitic, tuffaceous, and pyritic silty and shaly sediments.

The sediments of the Mt Isa Group, which are ~4,500 m thick, consist of a succession of dolomitic and siliceous siltstones and shales overlying a thin conglomeratic quartzite. They represent the latest infill of the Leichardt River Fault Trough^{1,10} of the North Australian Craton¹⁰. The sequence conformably and disconformably overlies some 5,000 m of continental flood basalts^{1,3} and associated shallow-water sediments. The strata of the Mt Isa Group dip to the west at angles of 60°–70° and are truncated at the top by the Mt Isa Fault³ (Fig. 1). They have undergone lower greenschist metamorphism and local deformation. Extensive field observations of the stratigraphic sequence comprising the Native Bee Siltstone, the Urquhart

Shale and the Kennedy–Spear Siltstone (Fig. 1) have recently been undertaken. We discuss those observations and their implications to ore formation at Mt Isa.

Algal stromatolites have been found both below the ore-bearing Urquhart Shale in the Native Bee Siltstone, and above in the Kennedy–Spear Siltstone. The stromatolites of the Native Bee Siltstone occur in laterally discontinuous chert–siltstone units averaging ~2 m thick and consisting mainly of stratiform sheets, but with some ridge–rill structures (Fig. 2d). The stromatolite fabric is smooth and parallel-laminated with small fenestrae. Associated with these structures are stromatolite fragments as either interbedded edge-wise conglomerates or accumulations in erosional rills. Fragments are frequently overgrown by stromatolitic mat. In places, teepee¹¹ structures up to 50 cm high disrupt the chert–siltstone sequence and there are local unconformities³ at the contact with the Breakaway Shale (Fig. 1).

The Kennedy–Spear stromatolites occurring between the A and B stratigraphic markers³ (Fig. 1) have different fabrics and morphologies from the Native Bee stromatolites. Domes up to 2 m in diameter and up to 80 cm high are most common, but there are also linear festooned ridges, ring structures, open rings and stratiform sheets. Most of these stromatolites are composed of branching and coalescing columns which have an average diameter of 0.5 cm and are up to 5 cm high. The closely spaced columns consist of a fabric of stacked hemispheroidal laminae. Laterally linked hemispheroidal and irregularly laminated fabrics are also present.

Within the Kennedy–Spear Siltstone channel deposits (Fig. 2c), flat-pebble conglomerates (Fig. 2b), and halite casts (Fig. 2a) occur together with stromatolites (Fig. 1). The channel sediments consist of coarse siltstones to fine sandstones. They contain large trough-shaped cross-beds with foresets up to 1.5 m long, dipping at 10°–30° to bedding, and showing bimodal palaeocurrent patterns.

The flat-pebble conglomerates consist of clast and matrix-supported fragments of either carbonate-intraclasts or stromatolitic chert or both. In the A-marker, pebbles are up to 20 cm long, rounded and often imbricated. Similar channels and flat-pebble conglomerates are also present in the lower formations (Fig. 1).

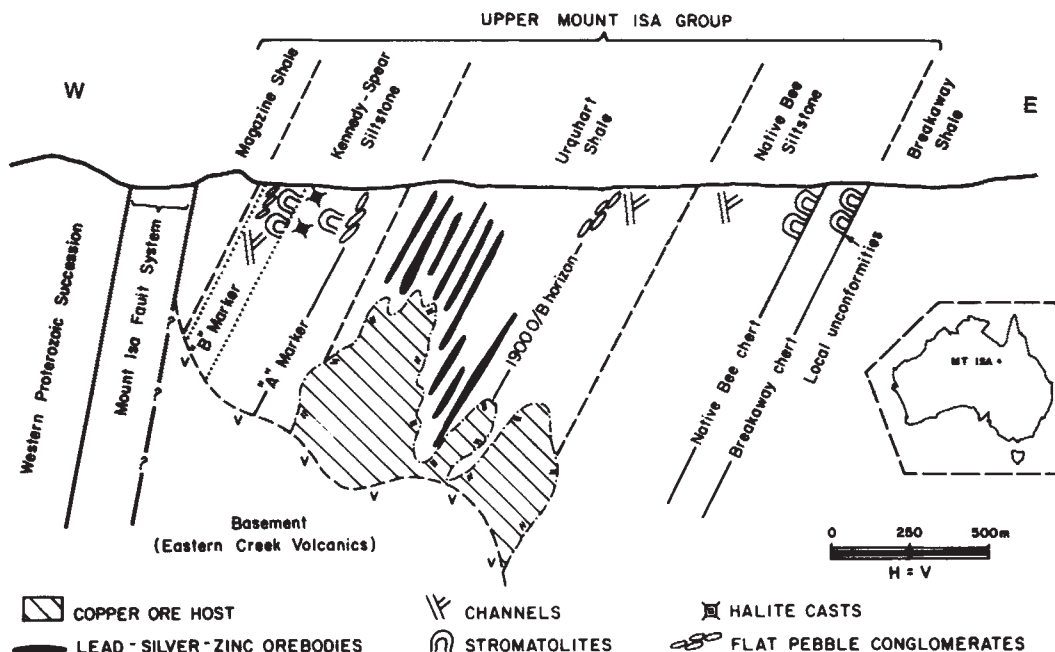
Following previous reports of pseudomorphs after sulphate evaporites^{5,6} occurring in the ore deposit, our recognition of halite casts stratigraphically above the deposit adds further evidence of hypersaline conditions in the depositional environment. Halite casts are present both in the form of 'hopper' cube moulds (Fig. 2a) and pagoda-type impressions on bedding planes.

Earlier reports from Mt Isa³ also mention the occurrence of small-scale cross-beds in the sequence. These are abundant throughout the Kennedy–Spear Siltstone, the Native Bee Siltstone and parts of the Urquhart Shale.

In the 1900 orebody horizon (Fig. 1), asymmetrical ripples occur together with centimetre-scale depositional cycles, carbonate-intraclasts and intraclast conglomerates. In upper parts of the Urquhart Shale, however, such ripples are confined to massive siltstone units (up to 10 m thick) which are intercalated with the thinly bedded Pb–Zn orebodies. Palaeocurrent data indicate a unimodal trend in contrast to that of the channels described above.

Table 1 lists the known sedimentary features from the Mt Isa sequence together with their environmental interpretation based on modern analogues. Shape and internal fabrics of the Mt Isa Group stromatolites closely resemble algal structures that occur today in shallow marine environments such as the tidal flats at Hamelin Pool, Shark Bay¹², and the Persian Gulf¹³. Similar stromatolites are also known from the shore of the Great Salt Lake, Utah¹⁴, and the ephemeral saline lakes of the Coorong area¹⁵. Most of the sedimentary structures associated with the Mt Isa stromatolites, for example the teepees, channels and flat-pebble conglomerates, independently provide evidence of shallow-water deposition. Teepee structures commonly

Fig. 1 Idealized cross-section (E-W) through the Upper Mt Isa Group formations hosting the Mt Isa Cu-Pb-Zn deposit. Inset: locality map.



fringe ephemeral lakes, and large cross-bedded channel structures are known from tidal¹⁶ and fluvial¹⁶ environments. Intracast flat-pebble conglomerates have been described from modern tidal flats¹⁶, shallow subtidal areas¹⁸ and ephemeral lakes¹¹.

Crystals of halite, gypsum and anhydrite occur in many evaporitic emergent and submergent environments¹⁹, and therefore their casts and pseudomorphs in the Mt Isa sequence indicate former evaporitic conditions, although they may not imply shallow water. Likewise small-scale cross-beds may occur at any depth.

However, the total of these sedimentary features, by the use of modern analogues, provides evidence of shallow-water deposition, intermittent subaerial exposure and hypersalinity during deposition of the Upper Mt Isa Group sediments. Such conditions are restricted to intertidal-supratidal marine or ephemeral lake environments. Unfortunately, few sedimentary structures are diagnostic of either of these two possibilities. However, the Mt Isa Group shows several stratigraphic features indicative of lacustrine sedimentation²⁰, such as the small-scale depositional cycles in the Urquhart Shale, the teepees in the Native Bee Siltstone and the complicated facies mosaic of

laminated organic, detrital and chemical sediments over large parts of the stratigraphic sequence. Therefore, we favour a non-marine depositional model with intermittent streams, playa-lake stages, flash-flood deposits, marginal ridge-rill structures and subsurface halite growth. As a modern analogue we suggest the Pleistocene to Recent lake complex of the Dead Sea Graben system²¹ including its continental sabkhas²². Our model is supported by previous sedimentary-petrographic data⁶ which indicates a hypersaline lake environment in an intramontane setting, and it is also consistent with the model of a fault-bounded basin as proposed for the Mt Isa Trough from evidence in pre-Mt Isa Group formations^{1,10}.

The recognition of shallow-water and hypersaline features in the Mt Isa Group sediments has significantly improved our understanding of the Mt Isa ore deposit. It is now evident that the mineralized sequence formed in a system with limited water depth. This clearly contradicts the belief that Mt Isa was a deep submarine trough. We therefore argue that the often postulated Red Sea submarine exhalative kind of model cannot apply to mineralization at Mt Isa, as it is inconsistent with the sedimentological evidence. At the same time any alternative syngenetic or early-diagenetic ore-formation model must conform

Table 1 Sedimentary features at Mt Isa and their environmental interpretation

Sedimentary features Upper Mt Isa Group				Environmental interpretation						
Unit				Water depth				Salinity		
				Emergent	Semi-emergent	Shallow	Deep	Hyper-saline	Pene-saline	Fresh to normal
Stromatolites	Native Bee Siltstone	Urquhart Shale	Kennedy-Spear Siltstone							
	X		X		X	X				
	X				X					
			X	X	X	X				
			X	X	X					
Evaporites		X	X						X	
		X							X	
			X					X		
Sedimentary structures	X	X	X		X	X				
	X	X	X		X	X	X			
	X		X	X	X	X				
	X	X	X	X	X					
	X		X	X	X					
	X			X						

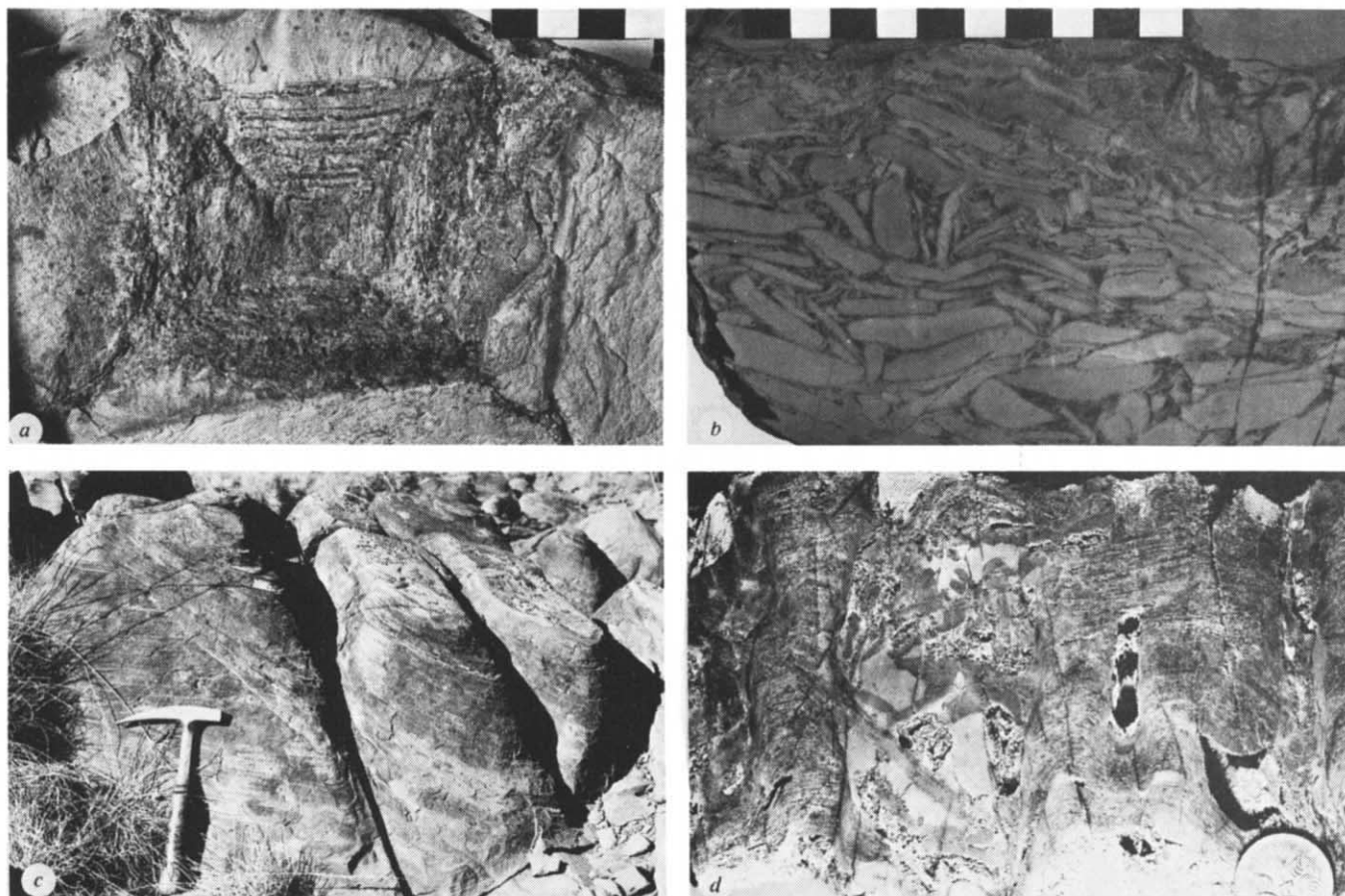


Fig. 2 Photographs of sedimentary structures in the Upper Mt Isa Group. *a*, Halite cast; note preferred growth along corners of former halite crystal, Kennedy-Spear Siltstone, B-marker, scale in cm. *b*, Flat pebble conglomerate with rounded intraclasts, Kennedy-Spear Siltstone, A-marker, scale in cm. *c*, Large trough-shaped cross-beds, Kennedy-Spear Siltstone, B-marker, hammer is 30 cm long. *d*, Stromatolitic ridge-rill-structures, Native Bee Siltstone. The basal LLH-S¹² stromatolite is overgrown on ridges by SH-V¹² columnar stromatolites. Structure on the right shows branching at intermediate growth-stage, then merging of columns, finally a new rill is formed on top of the structure. Note also chert fragments infilling the rill between this structure and the single SH-V column to the left. Coin is 1 cm in diameter.

to the physiochemical constraints²³ implied by shallow-water deposition. This point is strengthened by the presence of flat-pebble conglomerates in the 1900 orebody horizon indicating semi-emergent conditions in this part of the mineralized sequence. In addition, interpreted cryptagal features^{2,4,5} in the copper host, and the presence of stromatolites in horizons stratigraphically above and below the ore zone indicate similar environmental conditions elsewhere in the deposit. We, therefore, argue that the low hydrostatic pressure expected in such a depositional system would confine the possible range of volcanogenic-exhalative theories, permitting only the application of low temperature mineralization models at Mt Isa. On the other hand, the increasing evidence of hypersalinity in the stratigraphic sequence (1) supports the argument⁵ that evaporite-derived brines²⁴ were important to ore-formation at Mt Isa and (2) links the Mt Isa deposit closer to the group of stratiform base metal sulphide deposits that are associated with evaporitic sediments^{25,26}.

A more detailed account of the depositional environment of the Upper Mt Isa Group is in preparation²⁷ and investigations now focus on determining how significant the sedimentary environment was for the fixation of the metal sulphides. Although we consider the role of evaporites to be an important aspect of this we also appreciate that more evidence is needed before a complete ore-formation model can be formulated. However, we have now provided the geological framework in which such a model has to be placed.

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^{18}O modelling of freshwater inputs in Baffin Bay and Canadian Arctic coastal waters

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^{18}O -salinity modelling of marine environments has been proposed for various areas, including some Arctic seas. The present report concerns Arctic coastal waters collected along the west coast of Greenland, northern Baffin Bay, the Arctic archipelago and the north shore of Alaska. Measurements of ^{18}O versus salinity allow the identification of three main components: marine standard water, continental freshwater and sea ice meltwater. Continental waters may be represented by either glacial meltwater (western Greenland), land drainage (Mackenzie areas) or both. Quantitative determinations of the respective parts involved in mixed coastal waters imply a precise knowledge of the isotopic characteristics of the local continental waters. These determinations may be achieved either numerically or graphically.

As part of a research programme on glacio-marine environments, surficial (0–50 m depth) water samples were collected during the 1977–78 cruises of the *J. E. Bernier II*. The sampling sites were unequally distributed along the western Greenland coast, northern Baffin Bay, the Arctic archipelago and the northern coast of Yukon and Alaska (Fig. 1). Detailed investigation and sampling were performed in several fjords (Qaumarujuk, West Greenland; Cornelius Grinnell, Baffin Island; Ramah Bay, Labrador) during subsequent field trips and during the CSS *Dawson* cruise in Belle Isle Strait.

There have been many studies of the ^{18}O modelling of dilution effects, most involving a two-component model^{1–10}. Tan and

Strain¹¹ have proposed a three-component model for central Baffin Bay waters which introduces the sea ice meltwater component. Here we discuss the application of this model to coastal waters on a larger geographical scale. The examples presented below are restricted to areas where the various components of the coastal waters have been identified.

The Qaumarujuk Fjord (71°09' N, 51°13' W) is a 15-km long east-west embayment on the west coast of Greenland. It receives meltwater from several ice tongues of the Alfred Wegener glacier and is a classical example of homogeneous continental input, the glacial meltwater isotopic composition ranging from –20.5‰ to –21.7‰. A smooth horizontal and vertical dilution gradient is observed in the fjord (Fig. 2). At the surface, the ^{18}O values range from –16‰ at the outlet of the glacier to –6.3‰ at the mouth of the fjord. The ^{18}O content also increases very rapidly with depth, the $\delta^{18}\text{O}$ averaging –1.3‰ at 20 m depth and –0.8‰ at 50 m. In this case, the dilution of meltwater is essentially restricted to the surface. Figure 3a shows that the $\delta^{18}\text{O}$ -salinity relationship is almost perfectly linear between two components: the standard ocean water (salinity 34‰; $\delta^{18}\text{O}$ SMOW) and the glacial meltwater (salinity close to 0‰; $\delta^{18}\text{O}$ close to –20‰). Note that no deviation has been observed except for analytical errors.

The west Greenland current is responsible for the absence of sea ice early in July. This explains the simple two-component system which prevails in the Qaumarujuk Fjord. Moreover, recent precipitations have an average isotopic composition very close to that of the glacial meltwater¹² and thus do not affect the regression line.

The Cornelius Grinnell Fjord (63°20' N, 64°50' W) is located at the southeastern tip of Baffin Island. It is a narrow, north-south estuary, 2–7 km wide and 60 km long, which drains a small basin and has a small ice cap at its head. The ^{18}O -salinity relationship in the waters of the fjord varies according to the location of the sampling profile. The two-component system is observed upstream from the glacier outlet with a meltwater input (salinity 0‰; $\delta^{18}\text{O}$ –17‰) and a standard marine component (Fig. 3b). An effect of sea ice meltwater has been

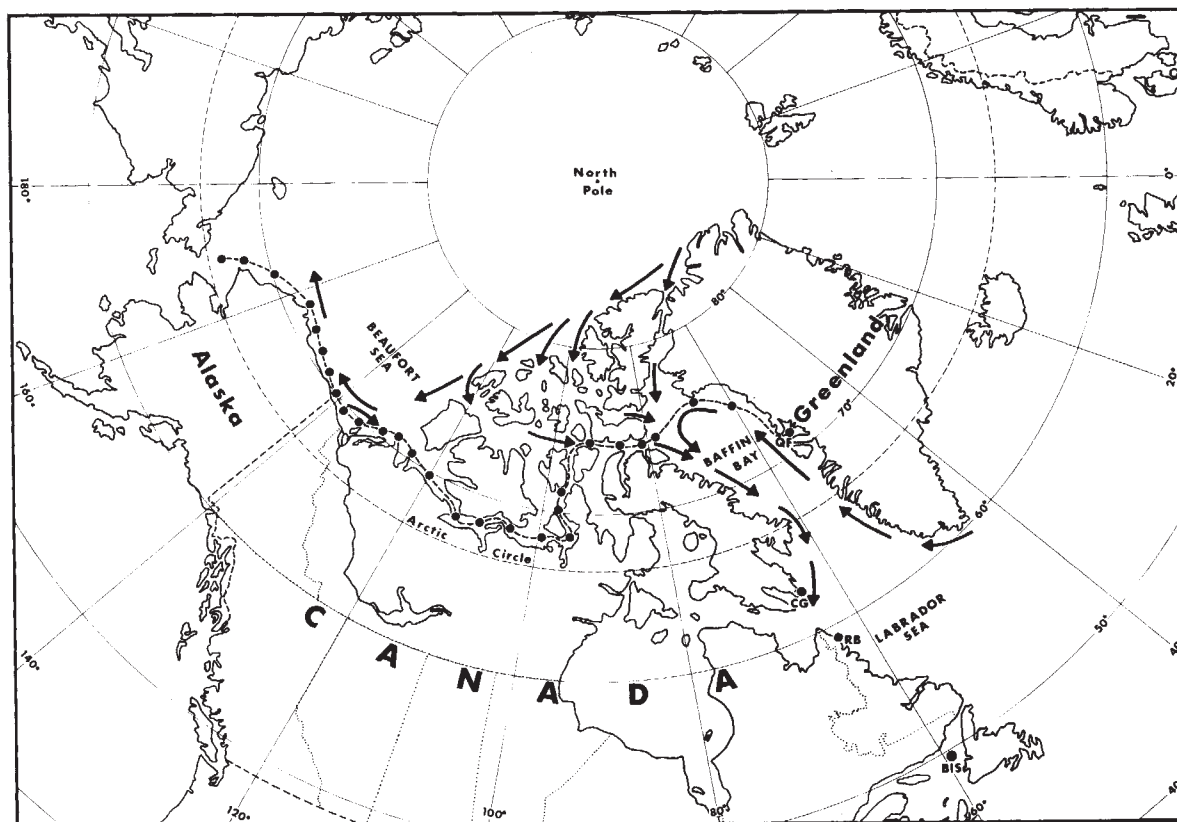


Fig. 1 Map of localization. ●, Water sampling locations during the *J. E. Bernier* cruise; letters indicate other stations: BIS, Belle Isle Strait; CG, Cornelius Grinnell Fjord; RB, Ramah Bay; QF, Qaumarujuk Fjord. Arrows represent dominant marine currents.

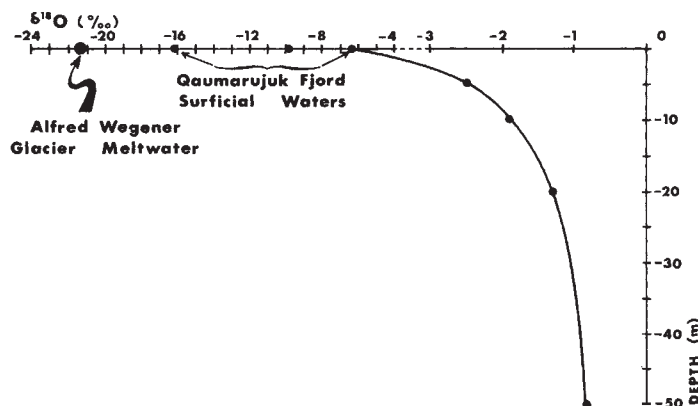


Fig. 2 $\delta^{18}\text{O}$ -depth relationship in Qaumarujuk Fjord surficial waters.

observed away from the glacier outlet, towards the open sea (Fig. 3c). This is due to the influx of oceanic water from the Canadian current in the estuary. Inasmuch as this site shows both mixing systems, it could represent the most frequent case at high latitudes.

The sampling programme in the Beaufort Sea along the coast of Yukon and Alaska was performed west of the main input of the Mackenzie River, but the pattern of the oceanic currents there (an anticyclonic gyre) causes a wide distribution of the continental signal (Fig. 1). Thus, the isotopic composition input is very close to -20‰ , which represents the average isotopic composition of the drainage basin of the Mackenzie River¹⁰. Some of the samples collected are quite representative of a theoretical double-component $\delta^{18}\text{O}$ -salinity relationship (Fig. 4), but most show a deviation towards higher $\delta^{18}\text{O}$ values, indicating an influence of sea ice meltwater.

The Arctic archipelago and northern Baffin Bay areas are quite similar, in terms of $\delta^{18}\text{O}$ tracing of the various components of the waters, to what has been observed in central Baffin Bay by Tan and Strain¹¹. The $\delta^{18}\text{O}$ -salinity values are scattered (Fig. 4). They reflect both a heterogeneous but discrete continental input and a strong influence of sea ice meltwater.

Figure 4 summarizes the two- and three-component models. Note first the amplitude of $\delta^{18}\text{O}$ or salinity changes of each pole. The marine component is certainly the most stable and in the experimental data presented above, extrapolation of the regression lines gives a salinity averaging 34.5‰ and a $\delta^{18}\text{O}$ within $\pm 0.7\text{‰}$. Surprisingly, the very restricted sampling area in comparison with the World Ocean volume still gives very similar values to those established for the SMOW by Epstein and Mayeda¹³. Deep Arctic water below 1,000 m, which could represent this component, effectively gives salinities around 34.9‰ and $\delta^{18}\text{O}$ values varying nonsignificantly from 0 to -0.7‰ (refs 14, 15).

The continental component shows large geographical variations in $\delta^{18}\text{O}$ content. Within the latitudinal range of our sampling ($50\text{--}70^\circ\text{N}$), the $\delta^{18}\text{O}$ values range from -17‰ to -21‰ . A clearer continental signal is observed with either a dominant glacial meltwater input (for example, West Greenland) or a large land drainage basin input (Mackenzie River). Within the area of the Arctic Islands, heterogeneous continental inputs may induce quite rapid shifts in $\delta^{18}\text{O}$ values. The absence of any record of the $\delta^{18}\text{O}$ content of precipitations makes it difficult to set absolute limits for the depletion in heavy isotopes of the meteoric waters above the Arctic Circle.

Finally, the sea ice meltwater component, which seems to be quite constant regionally according to the values measured by Tan and Strain¹¹, might be the most variable in time, with changes occurring from year to year in the relative contribution of frozen seawater and precipitations to the sea ice. Weeks and Lee¹⁶ observed a salinity ranging from 5.9 to 9.3‰ in sea ice of northern Baffin Bay, but lower values could be expected in sea ice originating from marginal marine environments. For instance, we observed salinities below 2‰ in southeastern

Hudson Bay sea ice where the seawater averages 15‰ . Due to the dispersion of broken ice by the currents, packs of ice could introduce anomalies in places. More or less proportional variations in $\delta^{18}\text{O}$ content of sea ice meltwater should occur.

It is easy to understand how the $\delta^{18}\text{O}$ -salinity changes of sea ice could be represented by a two-component model—from 100‰ frozen seawater with a $\delta^{18}\text{O} \sim +2$ or $+3\text{‰}$ (due to the water-ice fractionation) and a salinity slightly above 6‰ (ref. 11), to 100‰ frozen meteoric water, with a salinity of 0‰ and a $\delta^{18}\text{O}$ value equal to that of the local precipitations. Hence, the sea ice meltwater pole of the model should be restricted to the 100‰ frozen seawater, inasmuch as the continental pole includes any contribution of meteoric waters to the sea ice. Moreover, precipitation is very low in the Arctic, so that the actual characteristics of sea ice meltwater are close to the theoretical pole.

The relative contribution of each component to coastal waters, that is, standard marine water, sea ice meltwater and continental input, may be determined with the following equations, assuming a salinity equal to 0‰ for the continental water:

$$f_{\text{sw}} = \frac{S_{\text{sample}} + A(\delta^{18}\text{O}_{\text{sample}} - \delta^{18}\text{O}_{\text{cont}})}{S_{\text{sw}} + A(\delta^{18}\text{O}_{\text{sw}} - \delta^{18}\text{O}_{\text{cont}})}$$

$$f_{\text{si}} = \frac{S_{\text{sample}} + B(\delta^{18}\text{O}_{\text{sample}} - \delta^{18}\text{O}_{\text{cont}})}{S_{\text{si}} + B(\delta^{18}\text{O}_{\text{si}} - \delta^{18}\text{O}_{\text{cont}})}$$

where

$$A = \frac{-(S_{\text{si}})}{(\delta^{18}\text{O}_{\text{si}} - \delta^{18}\text{O}_{\text{cont}})}$$

$$B = \frac{-(S_{\text{sw}})}{(\delta^{18}\text{O}_{\text{sw}} - \delta^{18}\text{O}_{\text{cont}})}$$

and where f is fraction, sw is seawater, cont is continental water, si is sea ice meltwater, S is salinity and $\delta^{18}\text{O}$ is $\delta^{18}\text{O}/\text{SMOW}(\text{‰})$.

The contribution of sea ice meltwater may also be determined graphically by drawing a segment from the sea ice meltwater pole to the usual two-pole regression line through any datum point in Fig. 4. Thus, for sample JEB-4, $S=14.5\text{‰}$, $\delta^{18}\text{O}=-8.6\text{‰}$ and $f_{\text{si}}=0.16$, and for sample JEB-13, $S=30.3\text{‰}$, $\delta^{18}\text{O}=-1.2\text{‰}$ and $f_{\text{si}}=0.07$.

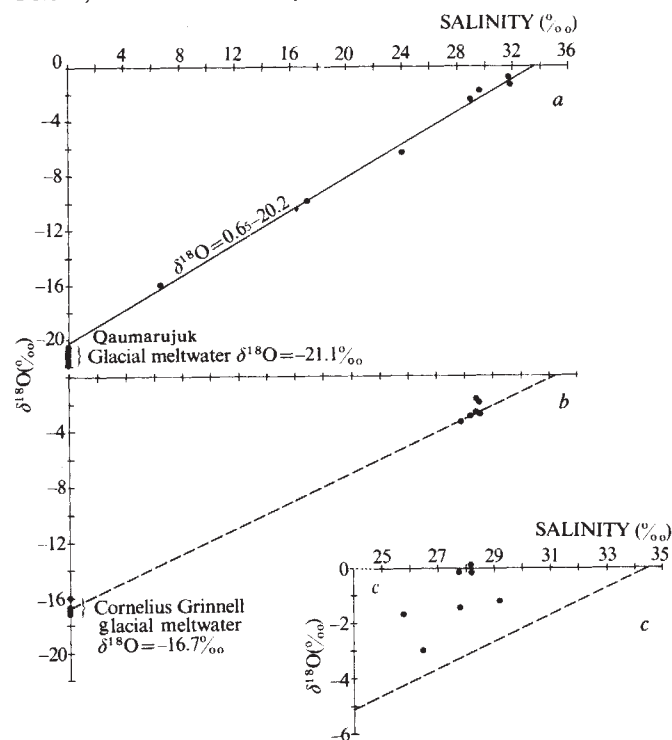
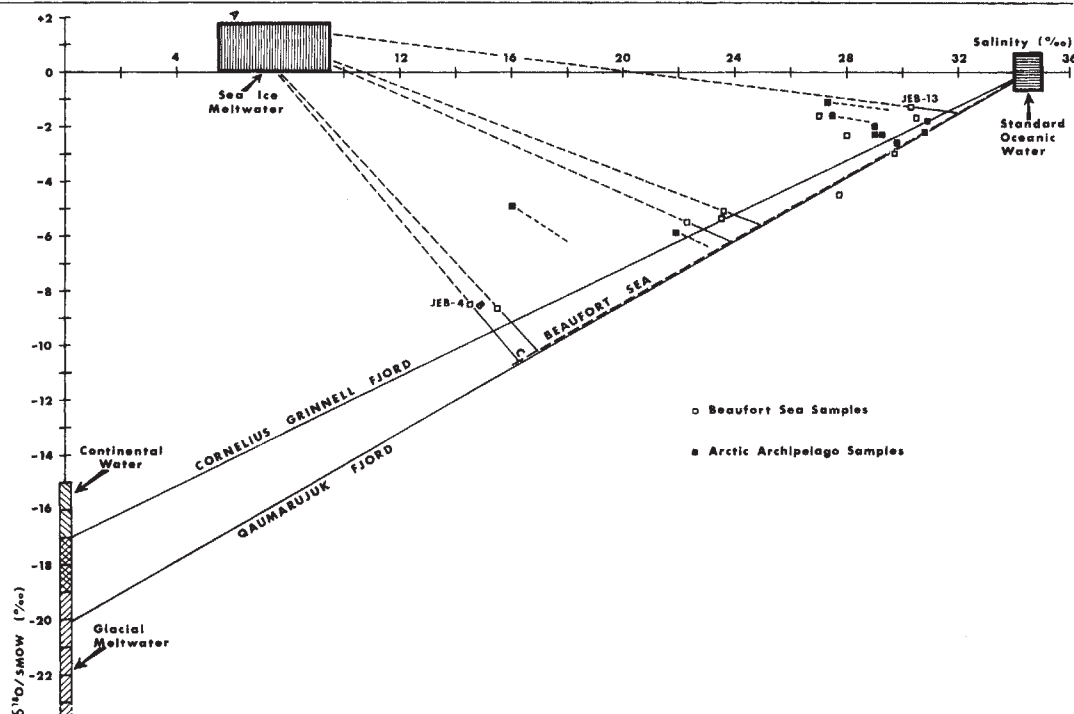


Fig. 3 a, $\delta^{18}\text{O}$ -salinity relationship in Qaumarujuk Fjord. The full line is the calculated regression line. b, c, $\delta^{18}\text{O}$ -salinity relationship in Cornelius Grinnell Fjord in the absence (b) or presence (c) of sea ice meltwater. The dashed line corresponds to an ideal two-component mixing system.

Fig. 4 Generalized $\delta^{18}\text{O}$ -salinity relationship with two- and three-component models, showing the $\delta^{18}\text{O}$ deviation with sea ice meltwater dilution.



Note that Tan and Strain¹¹ reduced these equations further by assuming an average, long-term continental signal, extrapolated from the $\delta^{18}\text{O}$ -salinity relationship in deeper waters. But this assumption clearly cannot be accepted for coastal waters because of the large variations in $\delta^{18}\text{O}$ content of continental waters.

The coastal waters analysed here show a dominant influence of standard oceanic water, which normally represents, out of the narrow fjords, more than 75% of the water masses. The continental input usually dominates the sea ice meltwater contribution. However, by means of the ice pack, sea ice meltwater may indeed be the major cause of $\delta^{18}\text{O}$ changes in surficial waters.

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Carbon isotope fractionation during microbial methane oxidation

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Methane, a common trace constituent of groundwaters, occasionally makes up more than 20% of the total carbon in groundwaters^{1,2}. In aerobic environments CH_4 -rich waters can enable microbial food chain supporting a mixed culture of bacteria with methane oxidation as the primary energy source to develop³. Such processes may influence the isotopic composition of the residual methane and because $^{13}\text{C}/^{12}\text{C}$ analyses have been used to characterize the genesis of methanes found in different environments, an understanding of the magnitude of such effects is necessary. In addition, carbon dioxide produced by the methane-utilizing bacteria can be added to the inorganic carbon pool of affected groundwaters. We found carbon dioxide experimentally produced by methane-utilizing bacteria to be enriched in ^{12}C by 5.0-29.6%, relative to the residual methane. Where methane-bearing groundwaters discharged into aerobic environments microbial methane oxidation occurred, with the residual methane becoming progressively enriched in ^{13}C . Various models have been proposed to explain the $^{13}\text{C}/^{12}\text{C}$ and ^{14}C content of the dissolved inorganic carbon (DIC) of

groundwaters in terms of additions or losses during flow in the subsurface^{4,5}. The knowledge of both stable carbon isotope ratios in various pools and the magnitude of carbon isotope fractionation during various processes allows geochemists to use the $^{13}\text{C}/^{12}\text{C}$ ratio of the DIC along with water chemistry to estimate corrected ^{14}C groundwater ages^{4,5}. We show here that a knowledge of the carbon isotope fractionation between CH_4 and CO_2 during microbial methane-utilization could modify such models for application to groundwaters affected by microbial methane oxidation.

Preliminary experiments⁶ indicated that ^{12}C was preferentially utilized by *Methanomas methanooxidans*, a methane-oxidizing bacterium. However, these experiments did not provide carbon isotope fractionation factors between CH_4 and CO_2 because, apparently, little CO_2 was produced. Methane-utilizing bacteria are very efficient converters of substrate carbon to biomass carbon—up to 80% conversion has been reported⁷.

Galimov⁸ concluded that carbon isotopic equilibrium between inorganic carbon and methane was generally attained in the "upper zone of sedimentary rocks", and was brought about by bacterially-mediated exchange reactions. Equilibrium conditions would require that the methane be depleted in ^{13}C with respect to the associated carbon dioxide. This has been verified in laboratory experiments⁹ and Richet *et al.*⁹ reported a theoretical value of ~0.68 for the fractionation factor between CH_4 and CO_2 ($\alpha_{\text{CH}_4-\text{CO}_2}$) at 25 °C. The fractionation factor, $\alpha_{\text{CH}_4-\text{CO}_2} = (^{13}\text{C}/^{12}\text{C})_{\text{CH}_4}/(^{13}\text{C}/^{12}\text{C})_{\text{CO}_2}$ and $1,000 \ln \alpha_{\text{CH}_4-\text{CO}_2} = \delta^{13}\text{C}_{\text{CH}_4} - \delta^{13}\text{C}_{\text{CO}_2}$.

The present study was designed to test Galimov's conclusions

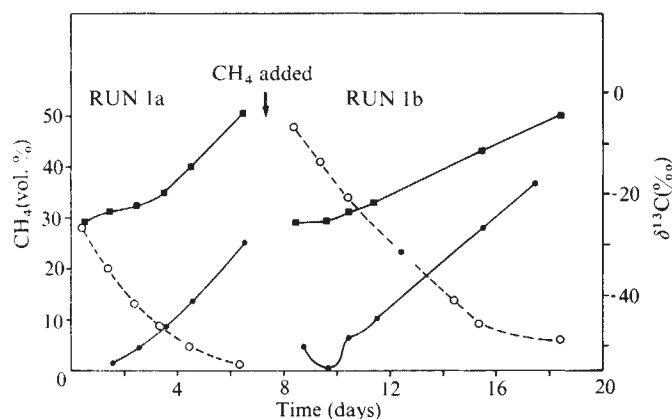


Fig. 1 The change in carbon isotopic composition of CH_4 (■) and CO_2 (●) as CH_4 is consumed by methane-utilizing bacteria. ○, CH_4 concentration.

by investigating mixed-cultures of methane-utilizing bacteria which yielded sufficient CO_2 to measure the carbon isotope fractionation between residual CH_4 and respired CO_2 . The mixed-culture was obtained from stream sediment by repeated harvesting of bacteria grown on the surface of a mineral salt medium incubated under a methane-air atmosphere. After purification, an inoculum of bacteria was washed into a column with about 2 litres of mineral salt medium. A $\text{CH}_4\text{-O}_2\text{-N}_2$ gas mixture was recirculated through the medium and bacteria. These experiments were carried out at $23 \pm 3^\circ\text{C}$. The CO_2 gas produced was continuously removed by passing the recirculating gases through a NaOH solution. Almost daily samples of the CO_2 and residual CH_4 were collected for carbon isotope analysis. The recirculating gas was also sampled for CH_4 , O_2 , N_2 analysis by gas chromatography. Enough $\text{O}_2\text{-N}_2$ was added to the system to maintain a constant, slightly positive pressure.

Figure 1 shows the changes in methane content of the recirculating gas and the $\delta^{13}\text{C}$ values for the produced CO_2 and residual CH_4 for one of the experiments. Standard deviations of $\delta^{13}\text{C}$ values were $<0.15\%$. The increasing $\delta^{13}\text{C}$ CH_4 values indicate the preferential utilization of the light carbon isotope with the expected reservoir effect shown by the increasing $\delta^{13}\text{C}$ CO_2 values. During this experiment the respired CO_2 was depleted in ^{12}C relative to the coexisting, residual CH_4 by 29.6% to 13.2% with the depletion diminishing as the CH_4 content of the recirculating gas decreased.

Figure 2 shows the variation in the isotopic fractionation factor, $\alpha_{\text{CH}_4\text{-CO}_2}$, for five individual experiments. A variation of $\alpha_{\text{CH}_4\text{-CO}_2}$ from 1.0313 to 1.0052 was found in these experiments. Thus, respired CO_2 was always depleted in ^{13}C relative to the residual CH_4 , but this depletion varied from 29.6 to 5.0%. This variation could not be consistently related to temperature, rate of CH_4 consumption or variations of the ratio CH_4/O_2 . Further experiments are planned to measure the fractionation factor in controlled growth conditions to try to relate isotopic fractionation to metabolic rate and/or environmental

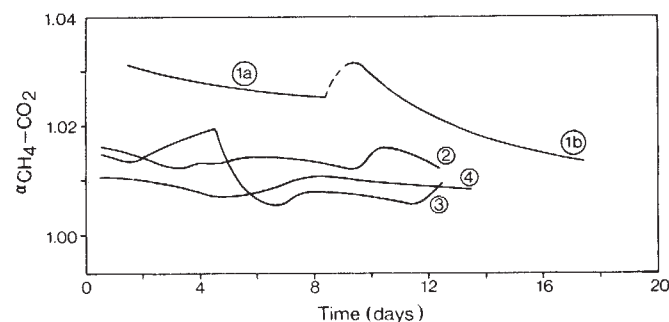


Fig. 2 The variation in $\alpha_{\text{CH}_4\text{-CO}_2}$ during microbial methane oxidation.

conditions. The preferential conversion of $^{12}\text{CH}_4$ to CO_2 is directly opposite to the enrichment of CO_2 in ^{13}C required to bring about carbon isotope equilibrium in the $\text{CH}_4\text{-CO}_2$ system. A review of carbon isotope measurements in the $\text{CO}_2(\text{DIC})\text{-CH}_4$ system where methane-producing bacteria are involved^{2,10-13} indicates that an equilibrium distribution of carbon isotopes is only occasionally approached. Therefore, bacterially-mediated reactions cannot be relied on to bring about isotopic equilibrium in the $\text{CO}_2\text{-CH}_4$ system in natural aqueous environments.

These experiments were conducted at higher temperatures and with higher nutrient concentrations than expected in most groundwaters and so quantitative application of the results to groundwater environments should not be made.

However, the experimental results were useful in understanding observations made on three groundwaters from shallow aquifers located on the north shore of Lake Erie, Ontario, Canada (Table 1). There, private wells are completed in carbonate bedrock or, in one case, in Quaternary deposits overlying the bedrock, but all are in regional bedrock groundwater discharge zones. They contained small, but temporally variable, quantities of CH_4 which range from 0.4 to 9 $\text{cm}^3 \text{CH}_4$ gas at STP per litre of water. The range of $\delta^{13}\text{C}$ CH_4 in one well was from -29.8 to -37.0% with the most ^{13}C -depleted value associated with the highest CH_4 content.

In this area biogenic methane dominates and always had $\delta^{13}\text{C}$ values below -50% . Underlying natural gas deposits contain methane with $\delta^{13}\text{C}$ values in the range -36.8% to -44.4% ¹⁴ and

Table 1 Concentration and carbon isotope ratio of methane collected from groundwaters in southern Ontario, Canada

Well	Sample date	Methane Concentration* (mol m ⁻³)	$\delta^{13}\text{C}$ (‰)
1	3 September 1975	0.16	-29.8
	20 April 1976	0.02	
	15 May 1976	0.08	
2	15 June 1976	0.26	
	30 September 1976	0.17	-28.8
3	3 September 1976	0.39	-37.0
	2 June 1976	0.10	-29.8
	26 June 1976	0.19	-33.4

* 1 mol $\text{CH}_4 \text{m}^{-3}$ is equivalent to $\sim 22 \text{ cm}^3 \text{CH}_4 \text{l}^{-1}$

such gas is occasionally found in the local groundwater. Thus, these three groundwater methanes have apparently not originated from either methanogenic activity or from direct, non-fractionating leakage from the underlying natural gas deposits. The observations can be explained if one assumes that the sampled groundwaters contained methane from natural gas deposits which had been partially oxidized by bacteria when the groundwaters entered more oxidizing environments. This process produced both temporally variable methane concentrations and carbon isotope ratios. When oxidation was more complete (low CH_4 contents) the methane was most depleted in ^{12}C and had the highest $\delta^{13}\text{C}$ CH_4 values. When methane oxidation was less significant, methane had a carbon isotope ratio similar to the methane found in natural gas deposits. Carbon isotope fractionation during migration of hydrocarbon gases is unlikely to produce more ^{13}C -enriched methane although preferential migration of $^{13}\text{CH}_4$ has been reported in molecular sieve columns¹⁵. Unfortunately the production of ^{12}C -enriched CO_2 during this process and its influence on the isotopic composition of the inorganic carbon pool cannot be demonstrated, in part because only small amounts of CH_4 seem to have been utilized, the bacteria convert a relatively small portion of this CH_4 to CO_2 and the aquifer material is carbonate from which considerable carbon has been derived. Where this process is quantitatively more significant, its effect on the carbon isotope geochemistry of the inorganic carbon pool in the

groundwater could be described by models^{4,5} after suitable modification. However, because of the difficulty in estimating the quantities of CH₄ converted to CO₂ (ref. 7) and the necessity of considering additional carbonate dissolution due to CO₂ production, such modelling is unlikely to yield unique descriptions.

It is essential to recognize biogenic methane oxidation when attempting to use stable carbon isotope ratios to define the origin of methane polluting groundwater supplies. For example, had the methane encountered in this field situation been biogenic in origin, with a $\delta^{13}\text{C}$ CH₄ of -60‰ for example, microbial oxidation could have left residual methane with an isotopic composition identical to methane from natural gas deposits found in this area (-36.8‰ to -44.4‰). An incorrect identification of such methane as leakage gas from reservoirs or distribution networks could have resulted. Where microbial

methane oxidation is possible, the carbon isotope ratio of the residual methane may not be a useful indicator of the origin of the gas. Other geochemical and isotopic parameters such as hydrogen isotopic composition, presence of higher hydrocarbons, must then be considered¹⁶. More refined experimentation is required before the controls of the biological isotope fractionation are understood and quantitative prediction of the carbon isotope ratio of respired CO₂ can be attempted for such field studies. Only then will quantitative treatment of the effect of methane utilization on the carbon isotope geochemistry and ¹⁴C dating of groundwaters be possible.

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Calvin-Benson cycle and sulphide oxidation enzymes in animals from sulphide-rich habitats

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The role of sulphide oxidation-driven production of reduced carbon in the nutrition of animals adapted to life in sulphide-rich habitats such as the deep-sea hydrothermal vents and intertidal mudflats has been a topic of recent interest¹⁻⁴. Chemoautotrophic sulphide-oxidizing bacteria have been isolated from samples of sulphide-rich vent water⁵⁻⁸, and it has been suggested that these could provide a food source for filter-feeding animals that live at the vents. The recent discovery of prokaryotic cells⁹ and activities of sulphide-oxidizing enzymes (which generate reducing power and ATP) and Calvin-Benson cycle enzymes¹⁰ within the trophosome tissue of the large vestimentiferan tubeworm of the vents, *Riftia pachyptila* Jones (Phylum Pogonophora)¹¹ suggests that sulphide-oxidizing chemoautotrophic bacteria exist in a symbiotic relationship with at least this vent species. This discovery led us to measure enzyme activities associated with sulphide oxidation, the Calvin-Benson cycle and nitrate reduction in a variety of other vestimentiferan tubeworms and bivalve molluscs which occur in sulphide-rich habitats. All the vestimentiferan worms and several of the molluscs were found to contain these enzymatic activities, suggesting that the putative animal-bacterial symbiosis first described in *Riftia pachyptila* may be of widespread occurrence in species living in environments offering simultaneous access to sulphide and oxygen.

The animals studied and important features of their habitats, including sulphide concentrations, are listed in Table 1 together with the measured enzymatic activities. The habitats included deep-sea rift vents (Galápagos Islands, 21°N and Guaymas Basin sites), fault vents (San Diego Trough), sewage outfalls,

shallow-water petroleum seepage areas, reduced sediments in offshore basins and intertidal mudflats. The enzymes studied were the two diagnostic enzymes of the Calvin-Benson cycle of net CO₂ fixation (ribulose-1,5-bisphosphate carboxylase, RuBPCase, EC 4.1.1.39; ribulose-5-phosphate kinase, EC 2.7.1.19), enzymes of sulphur metabolism (ATP-sulphurylase, EC 2.7.7.4; adenosine-5'-phosphosulphate reductase, EC 1.8.99.1; and rhodanese, EC 2.8.1.1) and nitrate reductase. We stress that this survey is qualitative in nature in that the specific activities listed in Table 1 should not be taken to represent *in situ* activities. Rather, our data must be regarded as suggestive of the capacities of these organisms to utilize the energy contained in sulphide for driving the net fixation of CO₂ and the reduction of nitrate to ammonia. That these enzymatic activities are likely to derive from symbiotic bacteria is implicit in the fact that, although the sulphur metabolism enzymes may occur in low levels in certain animal tissues for detoxification functions¹², the Calvin-Benson cycle enzymes and nitrate reductase are restricted to bacteria and plants. Symbiosis with photosynthetic organisms can be excluded, as all of the animals examined live in light-free habitats (Table 1).

All the vestimentiferan tubeworms contained one or more of the enzymatic activities, and in all cases the enzymes were found only in the trophosome, an organ which fills the greater share of the coelomic cavity of these worms. Trophosome of *Riftia pachyptila* contains prokaryotic cells at densities of approximately 10⁹ cells per g fresh weight of tissue⁹. Activities of RuBPCase in the freshest samples of trophosome available to us approach the levels found in fresh spinach leaves¹³. The lower activities found in other specimens could be either natural variation reflecting differences between individuals and habitats or artefacts of storage and preservation conditions.

Among the bivalve molluscs studied, all the hydrothermal vent bivalves, the clams *Calypotgena magnifica* and *C. pacifica* and the unnamed mytilid, exhibited activities of the enzymes measured. Thus, the aggregation of molluscs at hydrothermal vent sites may be supported, at least in part, by a nutritional strategy like that proposed for *Riftia pachyptila*^{9,10}. In these vent molluscs, as in the case of all bivalves in which these enzymes were found, activities were restricted to gill tissue. The gills of the enzyme-containing species were typically large, fleshy and dark in colour, and they contrasted sharply in appearance from the small, light-coloured gills of the bivalves which lacked these enzymes.

Table 1 Enzymes of the Calvin-Benson cycle, sulphur metabolism and nitrate reduction in marine animals from habitats in which sulphide and oxygen are present

SPECIES (PHYLUM)* AND HABITAT†	ENZYME ACTIVITIES				Nitrogen metabolism Nitrate reductase	
	Calvin-Benson cycle RuBPCase	Ru-5-P kinase	Sulphur metabolism ATP- sulphurylase	APS- reductase		
Rhodanese						
Rift vents						
<i>Riftia pachyptila</i> (Pogonophora)	0.22 ¹⁰	19.0 ¹⁰	74.0 ¹⁰	23.3 ¹⁰	7.6 ¹⁰	0.07
<i>Riftia</i> sp. (Guaymas Basin)	1.13		133.0	30.1	5.2	0.34
<i>Calypotgena magnifica</i> (Mollusca)	0.4					
Vent mytilid (unnamed) (Mollusca)	0.05					
Fault vent (San Diego Trough)						
<i>Lamellibrachia barhami</i> + unnamed species (Pogonophora)	0.4		30.0			
<i>Calypotgena pacifica</i> (Mollusca)	0.6		25.0			
Sewage outfall						
<i>Solemya panamensis</i> (Mollusca)	2.4	4.4	77.0	4.1	0.7	0.23
<i>Parvilucina tenuisculpta</i> (Mollusca)	0.01		3.8			0.08
Santa Barbara Basin						
<i>Lucinoma annulata</i> (Mollusca)	0.1	1.25	0.6		18.0	

Enzymatic activities are expressed as μ moles substrate converted to product per minute per gram wet weight of tissue at 25 °C. Enzyme abbreviations are: ribulose-1,5-bisphosphate carboxylase (RuBPCase), ribulose-5-phosphate kinase (Ru-5-P Kinase), adenylylsulphate reductase (APS reductase). Homogenate preparation procedures and enzyme assay conditions are as described in ref. 10, except for nitrate reductase²¹. Where no enzymatic activity is indicated, the assay failed to show the presence of the enzyme in all cases. Negative results may in most cases reflect lability of the enzymes.

* Other species examined in which none of the enzymatic activities was found were: Mudflat species: *Urechis caupo* (Echiura); *Glycera* sp. and *Tubifex* sp. (Annelida); *Macoma nasuta*, *Prothotaca staminea* and *Saxidomus nuttalli* (Mollusca). Petroleum seepage habitats: *Chaetopterus variopedatus* and *Myxicola* sp. (Annelida); *Kellia* sp. and *Lima hemphilli* (Mollusca). Sewage outfall: *Capitella* sp. (Annelida).

† Description of the rift vent habitat is given in refs 5–7. Sulphide concentrations of 160 μ M have been measured in mixed ambient and vented water^{6,7}. The fault vent (San Diego trough) is described in ref. 22. Sulphide concentrations at this site have not been measured. The sewage outfall sampled was near White's Point, California (near Los Angeles). Sulphide concentrations in the sediment from which *S. panamensis* and *P. tenuisculpta* were obtained range from 0.4 to 1.3 mM (ref. 15). In the Santa Barbara Basin, 'high' sulphide concentrations have been reported^{19,20}. Sulphide concentrations in mudflats of up to 8 mM have been reported^{1,2}.

Among the bivalve molluscs from non-vent sites, *Solemya panamensis* is of particular interest. Like other members of this genus¹⁴, *S. panamensis* has a greatly reduced gut (some congeners lack a gut entirely), and the nutritional strategy of these clams has been subject to controversy¹⁴. The discovery of enzymes associated with sulphide-driven chemoautotrophic metabolism within the gills of *S. panamensis* may help resolve this issue. In this regard it is noteworthy that a population of *S. panamensis* disappeared when changes in current patterns near the White's Point, California, sewage outfall led to oxygenation of the sediments, that is, to the disappearance of sulphide¹⁵.

Our failure to demonstrate these enzymatic activities in animals of sulphide-rich mudflats and petroleum seepage zones must not be taken as evidence for an absence of such chemoautotrophic systems in these habitats. We investigated only members of the macrofauna; the minute organisms of the meiofauna may possess the types of enzyme examined in this study^{1,2}.

It is extremely unlikely that the enzymatic activities found in the molluscs derive from bacteria trapped on the surfaces of the gills during filter-feeding. Other bivalve molluscs collected in the immediate vicinities of the enzyme-containing species, and presumably having access to the same exogenous food sources, did not contain any of the enzymes studied (Table 1). Furthermore, specimens of *S. panamensis* and *Lucinoma annulata* held for several weeks in surface seawater spiked with sulphide did not display reductions in the enzymatic activities. In the case of the vestimentiferan tubeworms, which lack a mouth, gut and anus, there seems to be little likelihood that trophosome bacteria could be trapped food bacteria.

The quantitative contribution of the sulphide-based chemoautotrophic metabolic activities, likely to be contained within symbiotic bacteria in all cases, to the nutrition of these animals remains to be established, as does the nature of the reduced carbon compound(s) supplied by the bacteria to animals. For *Riftia pachyptila* the identical ¹³C depletion values of trophosome and muscle, values which are strikingly different from the ¹³C depletion values of animals relying on photosynthetically fixed carbon, indicate a common pool of reduced carbon

compounds for the entire organism¹⁶. That is, the ¹³C data strongly suggest that carbon fixed in the trophosome is transported throughout the tissues of the worm. Nitrogen isotope data likewise suggest a common pool of reduced nitrogen in the different tissues of *Riftia*¹⁷. In the case of the bivalves which contain the enzymes of the Calvin-Benson cycle and sulphur metabolism, unusual ¹³C depletion values also have been found¹⁸ (values for *S. panamensis* are similar to those for the hydrothermal vent bivalves¹⁸; Rau, personal communication). Thus, for these bivalve molluscs as for the vestimentiferans, photosynthetically fixed carbon, obtained through feeding on particulate matter or via uptake of dissolved organic carbon, does not seem to be the major source of reduced carbon compounds. Rather, CO₂ fixed within the animals may play a significant, and perhaps dominant, role in the nutrition of these species. These species, in turn, may comprise a major share of the macrofaunal biomass in certain sulphide-rich habitats due to their abilities to exploit the sulphide and oxygen available in these environments. Bivalves and *Riftia pachyptila* are dominant members of the vent communities^{5,6}, and *Solemya panamensis* and *Lucinoma annulata* are major components of the macrofaunal biomass in their habitats as well (sewage outfalls¹⁵ and the Santa Barbara Basin^{19,20} respectively). In conclusion, some animals of sulphide-rich environments are not only able to tolerate these toxic habitats, but in addition are capable of exploiting the energy of sulphide to drive net CO₂ fixation and, thereby, reduce their dependence on ingestion of photosynthetically fixed carbon.

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Plummeting gannets: a paradigm of ecological optics

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Getting around the environment and doing things requires precise timing of body movements. Moreover, there is often little time available to pick up the visual information to organize the action. Consider, for instance, a batsman hitting a fast bowler or a bird alighting on a twig swaying in the wind. The visual and motor systems evidently work in close harmony, vision rapidly and directly providing the information for controlling the action. Here, we present evidence in support of a theory to explain how actions are visually timed. The evidence derives from a film analysis of the spectacular plunge dive of one of Britain's largest seabirds, the gannet (*Sula bassana*).

The theory is based on an analysis of the visual input considered as an optic flow field^{1,2}. When the organism is moving towards a surface, or an object is approaching, the time-to-contact under constant closing velocity is specified by a simple parameter, τ , of the optic flow field (see Fig. 1). The theory can explain, for instance, rapid timing of actions in ball games and how long jumpers regulate the cadence of their strides in zeroing in for take-off^{3,4}. But what if closing velocity is not constant? Can the theory also explain timing of actions in conditions of acceleration?

Watching gannets plunge diving near the Bass Rock in the Firth of Forth, it occurred to us that here was a good test for the theory. The dive is a beautiful example of finely timed locomotor activity. On sighting fish, the bird plummets down into the water from heights of up to 30 m, reaching speeds of up to 24 m s⁻¹ (54 m.p.h.). It first quickly assumes a swept-back-wing posture (Fig. 2a–c), which allows steering. Then, when only a split second from the water, it rapidly stretches its wings right back for a spearhead entry (Fig. 2d).

The gannet has to time its streamlining very precisely to avoid injury and so needs to keep track of its time-to-contact with the water. As this is a function of its height, velocity and acceleration, it might at first seem that the bird needs to monitor these three variables and compute the time-to-contact from them. However, it could control its timing in a simpler and more direct way by using the optical parameter τ (Fig. 1) which specifies time-to-contact under constant closing velocity.

We need simply consider the vertical component of the gannet's movement. If it starts from height Z_0 with zero velocity and dives with a constant acceleration A (assuming air resistance negligible over the speed range), then after time t its height $Z(t)$ and velocity $V(t)$ will be $Z(t) = Z_0 - At^2/2$ and $V(t) = At$, and so the optical parameter $\tau(t)$, which specifies $Z(t)/V(t)$, will have the value

$$\tau(t) = (t_d^2 - t^2)/2t$$

where $t_d = \sqrt{2Z_0/A}$ = duration of dive. Thus, the time-to-

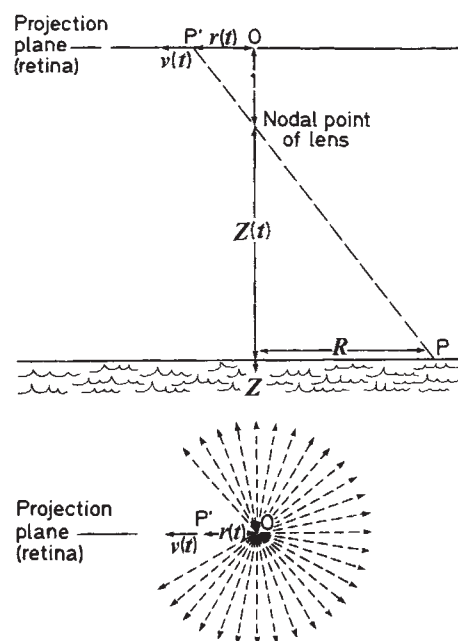


Fig. 1 How time-to-contact is specified in the optic flow field. The schematic eye is, at time t , at height $Z(t)$ and moving vertically downward with velocity $V(t)$ towards the water surface. Light reflected from the surface texture elements (for example, ripples) passes through the nodal point of the lens and projects an expanding optic flow pattern on to the retina. Considering an arbitrary texture element P and its moving image P' , then from similar triangles: $Z(t)/R = 1/r(t)$. Differentiating with respect to time: $V(t)/R = v(t)/r(t)^2$. Finally, eliminating R , $Z(t)/V(t) = r(t)/v(t) = \tau(t)$; that is, the time-to-contact under constant closing velocity is specified by the optical parameter $\tau(t)$. The optical geometry is similar for a slanting dive.

contact with the water is given by

$$\text{time-to-contact} = t_d - t = \tau(t) + t_d - \sqrt{\tau(t)^2 + t_d^2}$$

Now, although the optical parameter $\tau(t)$ does not alone precisely specify the time-to-contact, it could form the basis of a simple heuristic strategy. Suppose the gannet were to start streamlining an initiation time t_i after detecting that $\tau(t)$ had reached a margin value τ_m . It would therefore start when its time-to-contact with water was t_c , where

$$t_c = \tau_m + t_d - \sqrt{\tau_m^2 + t_d^2} - t_i \quad (1)$$

Figure 3 plots this equation for a particular pair of values of τ_m and t_i . It illustrates how following the ' τ strategy' would, in general, result in t_c increasing with t_d . Thus, the bird would have more time to streamline itself the longer its dive: the greater the risk of injury the larger the margin allowed for error.

By way of contrast, consider other strategies the bird might use and the form of the $t_c \times t_d$ curves that would result. First, suppose it could actually detect the time-to-contact and that it started streamlining when it reached a margin value; then the $t_c \times t_d$ graph would be a level line. Second, suppose it started streamlining when it reached a certain height. It would then have less time to prepare for entry the longer its dive, because it would be travelling faster; t_c would decrease as t_d increased. Finally, suppose it started streamlining either at reaching a certain (vertical) velocity or, which is mathematically equivalent, at a constant time t_s after starting the dive. The prediction would then be $t_c = t_d - t_s$; that is, t_c would increase linearly with t_d , the graph having a 45° slope.

To test between the hypothetical strategies, we used films of dives to measure how t_c varied with t_d . The films were shot from the stern of a trawler, fish having been thrown overboard to entice the gannets to display their skill. Their dives were consequently relatively short compared with those when they

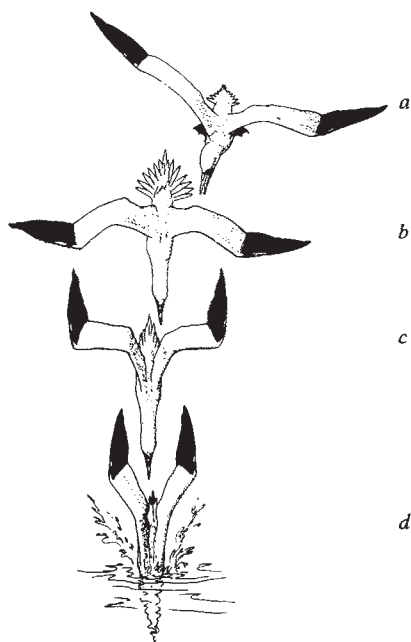


Fig. 2 Wing positions of diving gannet, *Sula bassana* (length ~0.9 m, wingspan 1.7 m). Illustration by John Busby. (Reprinted from ref. 7, courtesy of the author and publishers.)

are preying on a deep shoal of fish. We analysed 55 dives filmed at 50 f.p.s. or 150 f.p.s.

The results (Fig. 3) favour the τ strategy. The theoretical curve is the best-fitting member of the family represented by equation (1). It was computed minimizing the r.m.s. distance of the points from the curve; the minimized r.m.s. error was 49 ms. The birds were clearly not streamlining at a constant time-to-contact or at a constant height; nor do the data seem to follow a 45° line as predicted by the constant velocity and constant time-from-start strategies. Indeed, those strategies would make little sense for high dives because they would result in the birds maintaining steering capability for only short fractions of their dives. Clearly, strategies entailing detection of both height and velocity and subsequent computation can be formulated to fit the data. However, such strategies lack the simplicity of the τ strategy. Furthermore, the birds would probably not be able to detect their height and velocity with sufficient accuracy, given the very small degree of binocular parallax and the absence of invariant features in their field of view to furnish a spatial metric².

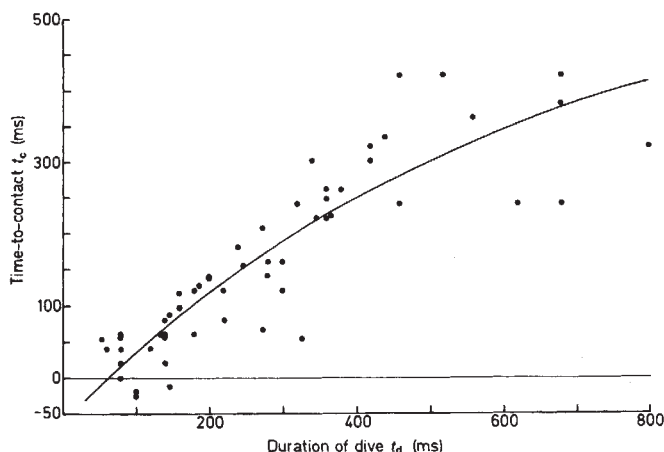


Fig. 3 How the time before contact when gannets start streamlining increases with the duration of the dive following the predicted curve of equation (1). Occasionally, streamlining was started after the head had entered the water (t_c negative), but it was invariably completed before submersion.

The parameter values of the curve in Fig. 3 provide measures of the average performance of the birds: t_i = action initiation time = 60 ms; τ_m = margin value of τ = 820 ms. It would be interesting to see whether gannets use the same margin value of τ in other activities—for example, in preparing to land on a cliff ledge. In that case, with approach velocity about constant, τ actually specifies the time-to-contact.

A further interesting question, of course, is whether people also follow a τ strategy in visually timing their actions in conditions of acceleration, as they seem to do in constant velocity conditions^{4,5}. We are now investigating the question by analysing performance on two skills: high diving and leaping to punch a dropping ball. There is also evidence suggesting that drivers control their deceleration by means of the optical parameter τ , for the time derivative of the parameter specifies whether or not their current braking force is adequate to stop before reaching the obstacle⁶.

It is beginning to appear that the information afforded by this ubiquitous optical parameter is much exploited by visual systems. The information is needed for many activities and is available to any seeing organism.

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Regulation of lymphatic contractility by arachidonate metabolites

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The circulation of lymph is apparently controlled partly by the pressure of fluid entering the lymphatic capillaries, aided by external forces such as skeletal muscle contraction and gut peristalsis^{1,2}, but lymphatic vessels themselves also contract rhythmically when distended *in vivo* or *in vitro* and this may be of major importance in the propulsion of lymph^{3–5}. We have studied the effects of biochemical mediators on isolated sheep and bovine lymphatic segments and on pressure pulses from indwelling lymphatic cannulae and report here that nanomolar concentrations of prostaglandin (PG) H₂ endoperoxide and a stable PGH₂ analogue elicited rhythmical contractions and increased the tone of isolated quiescent lymphatic segments. Some segments showed spontaneous rhythmic activity, and these spontaneous contractions could be inhibited not only by aspirin (a cyclo-oxygenase inhibitor) but also by inhibitors of thromboxane synthase (imidazole and compound UK 37248). The pulsatile pressure changes measured from indwelling lymphatic cannulae in conscious sheep were also suppressed by aspirin or imidazole infused directly into the lymphatic circulation. Our results suggest that endogenous thromboxane production is mainly responsible for spontaneous lymphatic contractions but that PGH₂ also has contractile activity. An unknown product of arachidonate and PGH₂, which could be formed non-enzymatically, inhibited both spontaneous and induced contractions.

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In quiescent lymphatic segments, nanomolar concentrations of PGH_2 increased the tone and elicited rhythmic contractions which decreased progressively in amplitude and frequency (Fig. 1a). The half life of PGH_2 in aqueous medium at 37°C is $\sim 5\text{ min}$. The stable endoperoxide analogue $15\text{-}(S)\text{-hydroxy-}11\alpha,9\alpha\text{(epoxymethano)-prosta-5-cis-13-trans-dienoic acid}$ (compound U46619) at threshold concentrations between 10^{-9} and 10^{-8} M increased lymphatic tone and induced rhythmic contractions that were maintained as long as the compound was present. Higher concentrations (up to 10^{-6} M) progressively increased the tone and eventually caused the vessel to go into spasm (Fig. 1b).

Administration of arachidonic acid (the natural substrate for PGH_2 formation) to quiescent vessels did not elicit any response, but when arachidonate was added to an actively contracting vessel there was usually a transient increase in tone, consistent with the concept of its conversion to PGH_2 ; following this, there was a suppression of contractions after a short delay (Fig. 1c). PGH_2 , when added at concentrations of $\sim 10^{-6}\text{ M}$ in the presence of U46619, always produced an increase in tone and in contraction amplitude, followed by complete inhibition after $\sim 5\text{ min}$ (Fig. 1d). These results suggested that an inhibitory product was formed from PGH_2 ; the stable prostaglandins D_2 , E_2 and $\text{F}_{2\alpha}$ (all metabolic products of PGH_2) were tested on rhythmically contracting lymphatic segments at concentrations up to 10^{-6} M but had no inhibitory effect. Prostaglandin H_2 is very unstable and decomposes spontaneously¹¹. When PGH_2 was incubated at 37°C for 15 min before adding to a lymphatic vessel already responding to U46619, inhibition of the contractile activity was observed within 1–2 min, indicating that the suppressor material was formed non-enzymatically.

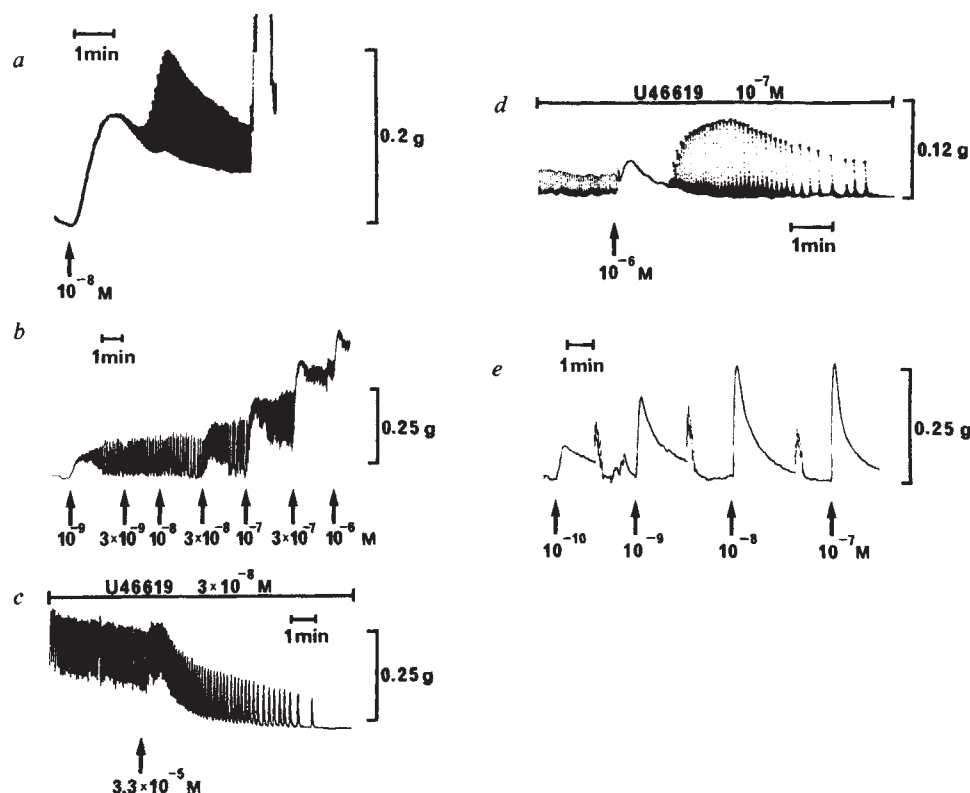
Several other potential mediators were tested on isolated lymphatic segments. Histamine, carbachol and prostaglandins

E_2 , $\text{F}_{2\alpha}$, D_2 and I_2 had no contractile effect at concentrations between 10^{-9} and 10^{-6} M . Bradykinin increased the tone but did not induce rhythmic contractions (Fig. 1e); these responses were transient and exhibited tachyphylaxis on repeated administration unless the vessel was extensively washed after each dose. Noradrenaline and serotonin induced modest rhythmic responses at concentrations $>10^{-7}\text{ M}$.

The results obtained with PGH_2 and compound U46619 did not establish unequivocally whether thromboxane or PGH_2 was the physiological stimulus that induced the rhythmic contractions. PGH_2 can be converted to thromboxane, and U46619, although resembling PGH_2 in structure, has been shown to act on thromboxane receptors¹². We therefore investigated the effects of imidazole and compound UK 37248, which are inhibitors of thromboxane synthase^{13,14}. These inhibitors were usually only partially effective against contractions induced by exogenous PGH_2 , although they suppressed the contractions completely in 4 of 13 preparations. These results indicated that PGH_2 has some lymphatic contractile activity in its own right (a conclusion supported by the immediate contractile response to PGH_2 ; see Fig. 1a, d), but they also suggested that the conversion of PGH_2 to thromboxane by the lymphatic vessel contributed to the endogenous regulation of lymphatic contractility.

To clarify the role of endogenous thromboxane we tested the effects of inhibitors on paired preparations of bovine lymphatic segments, one contracting spontaneously and the other induced to contract using compound U46619. Inhibition of cyclo-oxygenase with aspirin abolished the spontaneous contractions but had only a slight inhibitory effect on contractions induced by compound U46619 (Fig. 2a), and inhibition of thromboxane synthase with UK 37248 (Fig. 2b) and with imidazole (Fig. 2c) blocked spontaneous activity but had much less inhibitory effect

Fig. 1 Responses of isolated lymphatic segments to PGH_2 (a gift from Dr J. A. Salmon), compound U46619, arachidonic acid and bradykinin. The technique used to study lymphatic contractility *in vitro* was similar to that used for blood vessels¹⁹. Rings of sheep mesenteric lymphatics (5–10 mm long and $\sim 1\text{ mm}$ internal diameter) or bovine mesenteric lymphatics (5–10 mm long and $\sim 2\text{ mm}$ internal diameter) were excised and two wires inserted through the lumen. The lower wire was fixed to the bottom of a 4.0-ml perfusion bath and the upper was connected to an isometric transducer (UC₃ Gold cell) coupled to a chart recorder. The vessels were perfused with Krebs' solution of the following composition (in mmol l^{-1}): NaCl, 117; KCl, 5.7; MgSO_4 , 1.1; NaH_2PO_4 , 1.17; CaCl_2 , 2.54; NaHCO_3 , 25; glucose, 1.1. The Krebs' solution was aerated with 95% O_2 and 5% CO_2 and maintained at 37°C in a constant-temperature circulating unit. The vessels were placed under tension (0.3 g for sheep; 0.6 g for bovine lymphatics) and then allowed to equilibrate for 1 h before use. Preparations of bovine and sheep lymphatics responded consistently to all the contractile agents shown. Responses were complex, involving increases in tone and the induction of rhythmic contractions (except for bradykinin, which induced single contractions only). Because the parameters of tone, frequency and amplitude of contraction were differentially affected by the agonists, and the kinetics of the responses were complex and agonist specific (see, for example, a, b), representative recordings are shown, rather than dose-response curves for one of the parameters. The numbers of segments tested, all of which responded in the same manner as the recording shown, are given in parentheses below. Concentrations are given in mol l^{-1} and the additions of the agents described are indicated by arrows. a, Effect of PGH_2 on a quiescent bovine lymphatic vessel. The appropriate amount of PGH_2 (in acetone) was dried under nitrogen, made up in Krebs' solution and immediately added to the bath containing the lymphatic vessel. PGH_2 was washed out of the bath after 4 min to minimize non-enzymatic conversion to material that inhibits lymphatic activity, as described in the text ($n = 10$ sheep; $n > 30$ bovine segments). b, Cumulative effect of additions of compound U46619 (a gift from Dr J. E. Pike) to a quiescent sheep lymphatic segment ($n > 100$ sheep; $n > 50$ bovine segments). c, Effect of arachidonic acid on a sheep lymphatic vessel previously induced to contract rhythmically with compound U46619 ($n = 10$ sheep; $n = 3$ bovine segments). d, Effect of PGH_2 on a sheep lymphatic vessel previously induced to contract rhythmically with compound U46619 ($n = 10$ sheep; $n = 3$ bovine segments). e, Effect of bradykinin on a quiescent sheep lymphatic vessel. The preparation was washed three times after each addition of bradykinin ($n > 30$ sheep; $n = 9$ bovine segments).



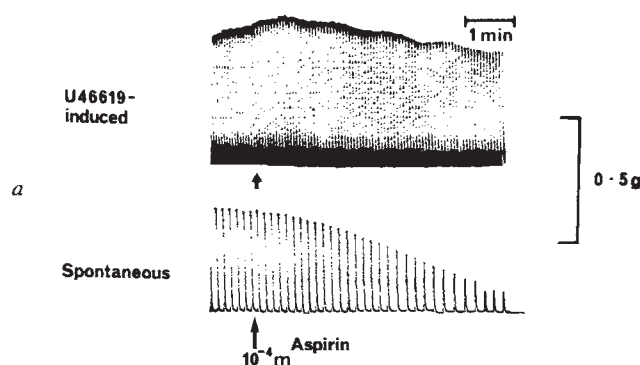
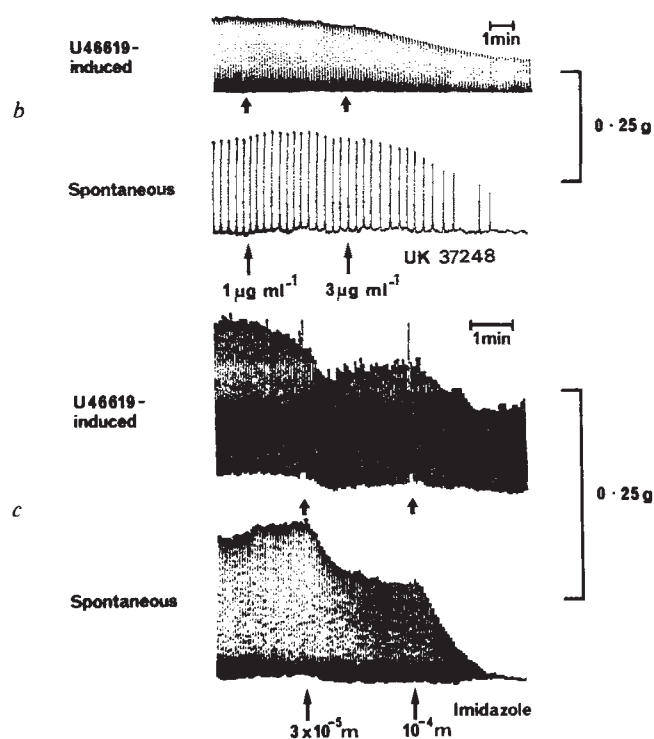


Fig. 2 Effects of cyclo-oxygenase inhibition and thromboxane synthase inhibition on lymphatic segments contracting spontaneously, or induced to contract using compound U46619 (10^{-8} M). The percentage of isolated segments that showed spontaneous rhythmic activity was initially low (10–15% of sheep vessels) but increased to >40% (of sheep vessels) as more experience was obtained with the preparation. This suggests that those vessels which did not show spontaneous activity may have been damaged during dissection; >70% of bovine lymphatics (which are larger and more muscular, and therefore presumably more resistant to trauma) showed spontaneous activity. Representative recordings are shown for bovine segments tested simultaneously, with the upper record in each experiment showing contractions induced by U46619 and the lower record showing spontaneous contractions. The numbers of replicate experiments performed (in all of which similar results were obtained) are given below in parentheses. *a*, Effects of cyclo-oxygenase inhibition by aspirin, 10^{-4} M ($n = 35$). *b*, Effects of thromboxane synthase inhibition by compound UK 37248, 4-(2-[1H-imidazol-1-yl]ethoxy)benzoic acid HCl, $1 \mu\text{g ml}^{-1}$ and $3 \mu\text{g ml}^{-1}$ ($n = 9$). *c*, Effects of thromboxane synthase inhibition by imidazole, 3×10^{-5} M and 10^{-4} M ($n > 50$).



on the U46619-induced contractions. The modest but detectable effects of these inhibitors on the contractions induced by compound U46619 suggests that contractile activity itself stimulated endogenous production of PGH_2 and thromboxane. In vessels that showed spontaneous rhythmic contractions, phentolamine (an α -blocker) or methysergide (a serotonin antagonist) had no inhibitory effect at concentrations up to 10^{-5} M. Very similar results were obtained with sheep lymphatic vessels. It therefore seems that the main endogenous mediators of lymphatic contractility in isolated vessels are arachidonate metabolites—in particular, thromboxane.

In conscious, unrestrained sheep, lymphatic pressure was monitored from indwelling cannulae in popliteal efferent

lymphatic vessels as described by Hall *et al.*⁴. The pressure pulses were rhythmic (Fig. 3*a*) and unrelated to respiration, heart rate or ambulatory movements. The infusion of aspirin into the lymphatic circulation via an indwelling afferent popliteal lymphatic cannula reduced the amplitude of the pressure pulses (Fig. 3*b*); pulse amplitude returned to control levels 40–90 min after aspirin infusion ended (Fig. 3*c*). Infusion of imidazole ($5 \times 10^{-5} \text{ mol min}^{-1}$ for 15 min) had a similar inhibitory effect, but the pressure pulses returned to normal 15 min after the infusion was stopped (data not shown).

There have been relatively few studies of the mechanisms regulating lymphatic contractility. The nervous system can apparently modulate the spontaneous activity of bovine

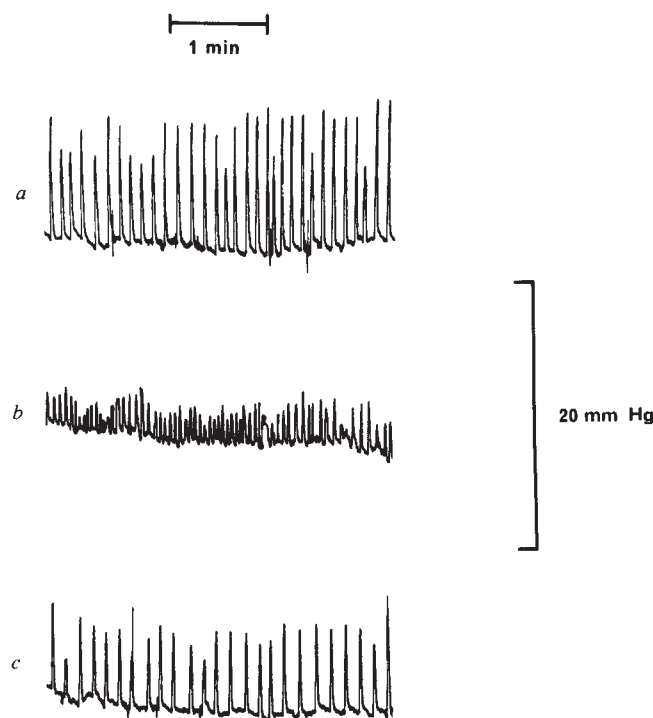


Fig. 3 Effects of direct infusion of aspirin into the lymphatic circulation on lymphatic pressure monitored by an indwelling lymphatic catheter in conscious sheep. Polyethylene cannulae were inserted under anaesthesia into one of the popliteal lymphatics afferent to the lymph node and into the vessel efferent to the node using methods described elsewhere²⁰. The preparation of the animal for the pressure measurements was similar to that described by Hall *et al.*⁴. The externalized cannula (Portex-PP50) from the efferent lymphatic was attached to a stainless-steel T-piece. One of the two remaining limbs of the T-piece was connected to 100 cm of cannula (same as above) which emptied into a plastic bottle containing 100 units heparin (Weddel Pharmaceuticals, UK). The bottle was positioned on a stand beside the metabolism cage at the approximate height of the point at which the catheter entered the lymphatic vessel in the animal. This catheter provided an arbitrary resistance enabling the pulse pressures to be monitored through the other arm of the T-piece. Pressure was monitored using a Statham P23 series pressure transducer coupled to a Devices M2 pre-amplifier and Oxford Instruments two-channel recorder (3000 series). Basal lymphatic pressures were highly consistent for several hours, provided the cannula remained patent and the animal did not change from a standing to a sitting position. Also, as shown in the figure, the individual pressure pulses in control conditions followed a regular, reproducible pattern. Infusions of aspirin (four animals) or imidazole (five animals) into the lymphatic circulation consistently reduced the amplitude of the pressure pulses by >50%, whereas infusions of the same volume of isosmotic saline in identical conditions had no effect. *a*, Control record. *b*, Aspirin was infused via the afferent lymphatic cannula into the lymphatic circulation while monitoring the pressure through the efferent lymphatic cannula. The aspirin was dissolved in homologous lymph plasma collected the day before the experiment. The record shows the pressure pulses 15 min after infusion of $2 \times 10^{-5} \text{ mol aspirin}$ (in 2 ml lymph plasma over a 6-min period). *c*, Record of pressure pulses 40 min after aspirin infusion.

lymphatic vessels¹⁵, but the generation of spontaneous contractions does not seem to be neurogenically controlled as tetrodotoxin does not inhibit this activity¹⁶. Earlier attempts to study the pharmacology of bovine mesenteric lymphatic vessels concentrated on agents such as noradrenalin, acetylcholine, serotonin and some of the prostaglandins¹⁷ but these studies did not reveal an obvious candidate for mediation of spontaneous lymphatic contractility. From our studies it seems that PGH₂ and thromboxane are important in the generation and maintenance of lymphatic contractions and that the lymphatic vessel itself can synthesize these compounds. The concepts of myogenic regulation and mediator control are not mutually exclusive: distension of lymphatics by increased intraluminal pressure could be the stimulus that initiates the release of PGH₂ and thromboxane from the vessels themselves. The formation of an inhibitor derived from arachidonate may also have some biological significance, particularly as lymphatic vessels could be affected by mediators entering the lymph from the interstitium—for example, prostanoids have been detected in high concentrations in lymph-draining inflammatory sites¹⁸.

The control of lymph flow is undoubtedly complex and many factors including extra-lymphatic forces will contribute to varying degrees dependent on circumstances and anatomical location. Although the relative importance of intrinsic contractility

and extralymphatic forces in lymph propulsion remains to be evaluated fully, biochemical mediators, particularly products of arachidonate metabolism, may be especially important in lymphatic regulation.

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Size-dependent variation of nodal properties in myelinated nerve

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Although the physiological properties of myelinated nerve fibres, such as action potential duration, are known to vary with fibre size^{1,2}, there has been little systematic examination of whether their biophysical properties show a similar variation (for example, in ionic conductance). Rather, differences have been identified between fibres on the basis of species^{3–6}, or on whether the fibres are sensory or motor^{7,8}. We report here the results of a comparison of amphibian (*Rana pipiens*) fibres of different sizes. Surprisingly, only the largest fibres (16–20 μm), usually selected for voltage-clamp study, had the large outward potassium currents described initially by Dodge and Frankenhauser⁹. Small fibres (9–11 μm) had little or no potassium conductance, and fibres of intermediate diameter had properties graded between these two extremes. This gradual loss of potassium conductance in fibres of decreasing size seemed to be due to the progressive electrical concealment of the potassium channels beneath the paranodal myelin. Thus brief treatment with the demyelinating agent lysophosphatidyl choline^{10,11} induced a large potassium conductance in the smaller fibres, but had little effect on the ionic currents of large fibres. We conclude that the biophysical properties of myelinated nerve fibres can vary with fibre size.

Single myelinated nerve fibres, 8–20 μm in diameter, were dissected from the sciatic or tibial nerves of 3–3.5-inch northern *R. pipiens* and immediately mounted in a standard Vaseline-gap voltage-clamp chamber^{9,12}. The action potential was recorded and, where possible, its shape was used to judge whether a particular fibre was sensory or motor^{13,14}. The fibre was then examined under voltage-clamp by established methods^{9,12,15–17}. Leakage current was subtracted by appropriate analogue circuitry (the leak conductance averaging $0.21 \pm 0.04 \times 10^{-7} \text{ S}$ in the largest fibres), and the resultant membrane currents were recorded digitally with 20- μs resolution for step depolarizations of 40–180 mV in 10-mV increments. Sodium and potassium currents were recorded simultaneously to ensure that any systematic changes in internodal resistance, which would alter the absolute value of the current measured, would not differentially affect either the sodium or potassium current. Fibres were

held at $\sim -90 \text{ mV}$ by the criterion that steady-state sodium inactivation (h_{∞}) has a value of 0.7 at -77 mV (ref. 18). The complete $h_{\infty}(V)$ curve was measured for each series of depolarizing voltage steps and used to ensure a constant holding potential. After recording, the diameter of the nerve fibre was measured in the voltage-clamp chamber using a compound microscope.

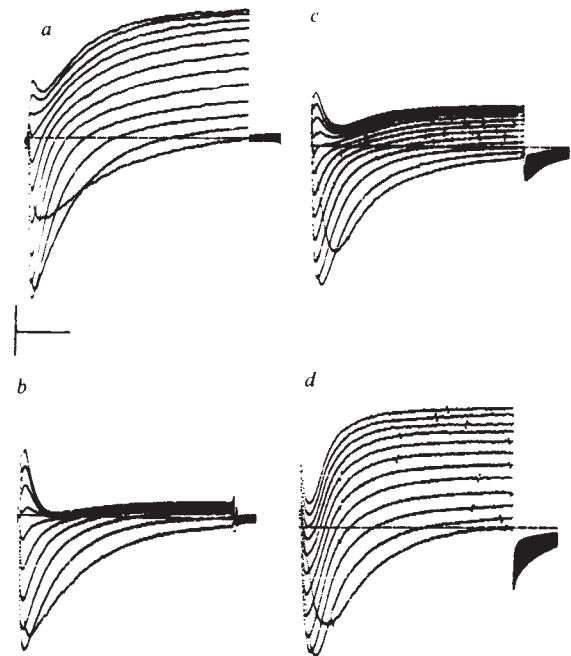


Fig. 1 Membrane currents from three myelinated fibres of *R. pipiens*. *a*, 17.5 μm ; *b*, 9 μm ; *c*, 12 μm . All recordings were made in tetraethylammonium-free Ringer's. Note the increasing relative amount of delayed outward current with increasing fibre diameter. Records in *d* are from the same nerve fibre as *c*, but were obtained 2 h later. During this period the paranodal myelin retracted, as shown by an increase in the nodal capacitance. This increase was accompanied by an increase in K^+ current in the face of stable sodium currents. Holding potentials were -80 mV (*a*), -82 mV (*b*) and -85 mV (*c*, *d*). Currents during step depolarizations in 10-mV increments are shown beginning at -38 mV (*a*), -32 mV (*b*), -35 mV (*c*, *d*). Calibrations are 2 ms and 16 nA (*a*), 8 nA (*b*), or 12 nA (*c*, *d*). Absolute currents were calculated using measurements of internodal resistance as a function of fibre diameter¹⁹. Temperature, 11–14 $^{\circ}\text{C}$.

Peak inward sodium currents and steady-state potassium currents were measured as a function of membrane potential and converted to conductances rather than permeabilities⁹. Potassium chord conductance was calculated as $g_K(V) = I_K(V)/(V - E_K)$, where E_K is the potassium equilibrium potential. Peak sodium conductance was determined using an approximation of the constant field equation appropriate for normal Ringer's¹⁸. There was no change in E_{Na} (sodium equilibrium potential) as a function of fibre diameter. Maximum sodium and potassium conductances ($\bar{g}_{Na}$ and $\bar{g}_K$), as well as the ratio $\bar{g}_K/\bar{g}_{Na}$, were calculated from the experimental data obtained for $V \geq +20$ mV.

The data show that while both the maximum sodium and potassium conductances increased with fibre diameter, the increase in the potassium conductance was much greater. Thus, whereas $\bar{g}_{Na}$ increased by a factor of 5 ($0.94 \pm 0.18 \times 10^{-7}$ S to $5.06 \pm 1.81 \times 10^{-7}$ S) as fibre diameter increased from 9 to 20 μ m, $\bar{g}_K$ increased by a factor of 35 ($0.04 \pm 0.01 \times 10^{-7}$ S to $1.46 \pm 0.50 \times 10^{-7}$ S). The conductances for large fibres agree with previous observations^{5,19}. The effect of these changes on the sodium and potassium currents is shown in Fig. 1a, b. The records in Fig. 1a were obtained from a 17.5- μ m fibre and have the large potassium currents previously observed in amphibian nerve, whereas those in Fig. 1b were recorded from a small fibre (9 μ m) and show very little delayed potassium current. As the exact changes in nodal area with fibre diameter are not known for *R. pipiens*, and as both $\bar{g}_{Na}$ and $\bar{g}_K$ can vary substantially from one fibre of a given size to another, the dependence of $\bar{g}_K$ on diameter is best illustrated by normalizing to $\bar{g}_{Na}$ (Fig. 2). The ratio $\bar{g}_K/\bar{g}_{Na}$ is 0.05 ± 0.01 for 9–11- μ m fibres, and then increases almost linearly for fibres 12–20 μ m in diameter, reaching a maximum of 0.28 ± 0.02 in the largest fibres.

It is appropriate to consider whether mechanical damage could have resulted in these findings, perhaps by causing an increase in internal sodium concentration and thus an inhibition of the potassium conductance²⁰. Several lines of evidence argue against this or other possibilities. First, the fact that both large and small fibres had similar values for E_{Na} and E_K indicates that there was no selective damage of small fibres. Second, it is difficult to imagine how, in small fibres, a potassium conductance could be lost due to trauma, without g_{Na} , generally more labile, decreasing by an equal or greater amount. Furthermore, in practice, trauma usually results in an increase in apparent $\bar{g}_K$ (ref. 5 and our unpublished observations). Finally, the presence of large potassium conductance in normal, undissected (and therefore undamaged) large amphibian fibres was demonstrated by the fact that 4-aminopyridine, which blocks the potassium channel, increased the duration of the extracellularly recorded action potential. In mammalian fibres g_K is low; thus 4-aminopyridine has no significant effect²¹.

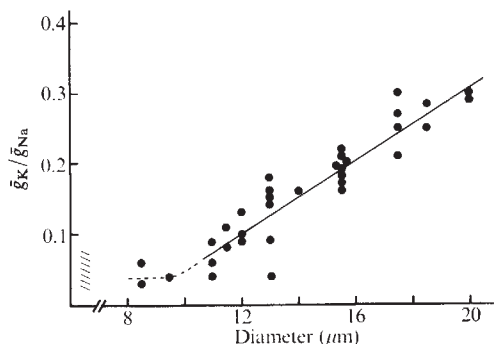


Fig. 2 Ratio of maximum potassium conductance to maximum sodium conductance as a function of fibre diameter. The fibre diameters are the means of diameters measured in the A and B pools of the voltage clamp chamber^{9,12}. Although the measurements of fibre diameter are proportionally accurate, they may differ by 1–2 μ m from the absolute diameter because of refractive errors. The lines at the far left of the figure illustrate, for comparison, the range of conductance ratios recorded in rat myelinated fibres of 9–11 μ m diameter.

We have also examined fibres from rat sciatic nerves and found $\bar{g}_K/\bar{g}_{Na}$ ratios of only 0.04–0.07, in good agreement with values from other studies of mammalian fibres^{4,5,22}. Rat nerve fibres are smaller than frog; those examined were between 9 and 11 μ m. These fibres had an average $\bar{g}_{Na}$ of $1.36 \pm 0.15 \times 10^{-7}$ S and an average $\bar{g}_K$ of $0.06 \pm 0.02 \times 10^{-7}$ S. Note that such small fibres in *R. pipiens* have similarly small relative potassium conductances, and thus the absence of potassium currents in mammalian fibres^{3–5,10,11} may be, at least in part, due to their different size. Similarly, it is possible that certain other dissimilarities in fibre properties, thought to be species dependent^{5,6}, may be related to systematic differences in the diameters of the fibres examined.

Although we could not designate all the amphibian fibres examined as sensory or motor on the basis of their action potential configuration^{13,14}, this was possible for most fibres. Those judged to be sensory ranged from 11 to 17.5 μ m in diameter, while those judged to be motor had diameters of 9–15.5 μ m: although the $\bar{g}_K/\bar{g}_{Na}$ ratios of such fibres showed no consistent differences, this conclusion must be regarded as tentative in the absence of a more definitive identification of fibre type.

Although nerve fibres from *R. pipiens* have progressively lower potassium conductance as fibre diameter decreases, the potassium conductance in smaller fibres can be dramatically increased by procedures which disrupt the paranodal myelin. The transient addition of lysophosphatidyl choline (LPC) to the node has this effect in rabbit and rat fibres^{10,11,16}. In a 9 μ m frog fibre we found that treatment with 1 mg ml⁻¹ LPC for 1 min increased $\bar{g}_K$ from 0.03×10^{-7} S to 0.21×10^{-7} S, but did not affect the sodium conductance. This increase in g_K was abolished by gallamine triethiodide (Flaxedil), a potent blocker of the potassium channel^{10,16}. Such an increase in $\bar{g}_K$ was not seen after LPC treatment in large fibres, and in small fibres the value of $\bar{g}_K/\bar{g}_{Na}$ after LPC treatment usually resembled that observed in untreated normal large fibres. Thus the ratio of the sodium and potassium channels present in the combined nodal and paranodal membranes may be similar in large and small fibres. To exclude the possibility that LPC may acutely affect the nodal axolemma²³, we also allowed the paranodal myelin to retract spontaneously with time. This effect is illustrated in Fig. 1c,d for a fibre 12 μ m in diameter. The records in Fig. 1d were obtained 2 h after those in Fig. 1c and show a significant increase in maximum potassium conductance ($\bar{g}_K$ increased from 0.12×10^{-7} S to 0.36×10^{-7} S), but no change was observed in the sodium currents ($\bar{g}_{Na}$ of 0.96 and 1.00×10^{-7} S, respectively). The increase in potassium conductance was accompanied by an increase in nodal capacitance, indicative of an increase in apparent nodal area.

Thus the nodes of small *R. pipiens* fibres behave like those of rabbits and rats^{10,11} in that paranodal disruption does not increase the sodium conductance, but significantly increases the potassium conductance. It therefore seems that, as at rabbit nodes¹⁰, the sodium and potassium channels in small *R. pipiens* fibres are segregated, with the sodium channels concentrated at the nodal axolemma, and bounded on at least one side by an abundance of potassium channels. It is possible that the sodium and potassium channels do not intermingle in large fibres either, but remain spatially segregated in myelinated fibres of all diameters. Whether or not this is the case, it seems that in *R. pipiens* at least, as fibre diameter increases so progressively more potassium channels are revealed, either as a result of their effective relocation into the nodal membrane, or perhaps due to a size-dependent change in the paranodal seal resistance.

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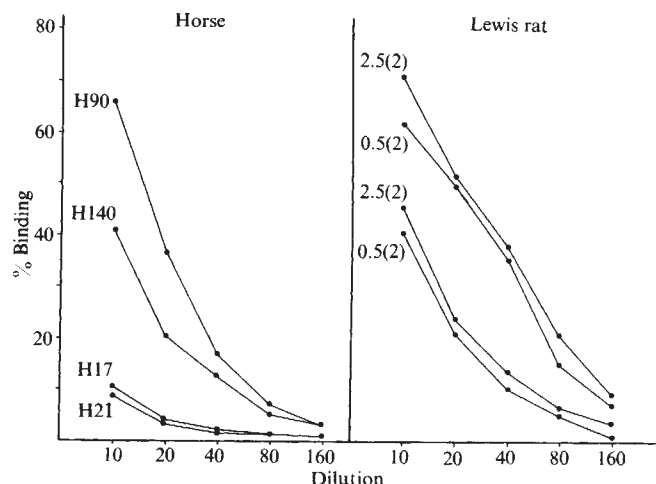


Fig. 1 Titration of antibodies to P_2 in horses with NCE. Binding of $^{125}\text{I}-P_2$ in the pellet is expressed as a percentage of the total counts added (10^4 c.p.m.). The four horses are identified by their numbers (H17, H21, H90, H140). Each titration curve for the rat sera is identified by the dose of bovine peripheral myelin received and the maximum neurological score in brackets. Thus 2.5(2) indicates that this animal received 2.5 mg myelin in Freund's complete adjuvant and developed a maximum neurological score of 2 (limp tail, mild or moderate paraparesis). All rat sera were obtained 21 days post immunization.

Circulating antibodies to the neuritogenic myelin protein, P_2 , in neuritis of the cauda equina of the horse

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The syndrome known as neuritis of the cauda equina (NCE) of the horse is an uncommon condition in which a chronic polyneuritis affects especially the sacral and coccygeal nerves. This leads to paralysis of the tail, rectum and bladder, and a loss of sensation in the sacral dermatomes with a surrounding zone of hyperaesthesia. It is frequently associated with cranial nerve paralyse, particularly of the trigeminal and facial nerves. The aetiology of the condition is unknown¹, but similarities between this condition and the Guillain-Barré syndrome in man and experimental allergic neuritis (EAN) have been noted^{1,2}, and it was this that stimulated our interest. Bovine P_2 , a protein purified from peripheral myelin and having a molecular weight of approximately 15,000, has been shown to be the neuritogenic antigen in EAN^{3,4} and animals with EAN have circulating antibodies to P_2 ⁵. We report here that four horses with NCE all had circulating antibodies to P_2 .

In the early stages of NCE, the nerves may show no macroscopic abnormality or may be more translucent than normal, and in histological sections there is demyelination and infiltration with lymphocytes, plasma cells and macrophages. In more advanced lesions the affected nerves are enlarged and firm due to fibrous thickening of the epineurium in particular, and the nerve fibres are degenerate and infiltrated with mononuclear cells, together with occasional multinucleate giant cells. Both early and advanced lesions may be found in different fascicles of the same nerve or in different nerves of the same horse, whilst other peripheral nerves are apparently unaffected.

The four horses investigated here (H17, H21, H90 and 140) were diagnosed as definite cases of NCE on clinical signs and on gross postmortem and histopathological grounds. The salient features of each case are listed in Table 1. The horses were investigated at the Royal Veterinary College over a period of 4 yr; blood samples were collected at clinical examination and sera stored at -70°C . Control sera had been stored at -20°C for 12–15 months. Antibody to P_2 was assayed by a modification of the method of Sarvas *et al.*⁶. Aliquots (50 μl) of appropriately diluted test serum were incubated overnight at 4°C with 10 μl ^{125}I -bovine P_2 ($\sim 10^4$ c.p.m.) in a total volume of 500 μl . Immune complexes were isolated by the addition of 600 μl 18% polyethylene glycol followed by incubation for 30 min at 4°C and centrifugation. The radioactivity in the pellet was expressed as a percentage of the total counts added. In some experiments 50 μg of myelin protein (prepared by standard techniques^{3,7}) were added in a volume of 50 μl just before the addition of radiolabelled P_2 , keeping the total incubation volume constant. Male inbred Lewis rats were immunized with bovine peripheral myelin as described previously³, the sera being obtained 21 days after immunization. Sera from 10 clinically normal horses were used as controls and these bound $5.0 \pm 1.8\%$ of the added radiolabelled bovine P_2 (range 3.1–7.8). The sera from all four neuritis cases bound a significantly greater amount of the label than did the controls, ranging from $8.1 \pm 0.8\%$ for H21 to $65.0 \pm 1.1\%$ for H90 (Table 2).

Bovine P_2 (50 μg) caused a reduction of binding to control levels in all four cases, as well as slight but not significant

Table 1 Case histories of the four horses with NCE used in this study

Horse no.	Age/sex	Time from onset of neurological signs to serum withdrawal	Clinical signs of cranial nerve involvement	Lesions at postmortem examination
H17	8-yr old mare	39 days	No	Marked advanced lesions of cauda equina. No macroscopic lesions of cranial nerves.
H21	2-yr old mare	12 months	No	Slight enlargement of nerves of cauda equina; mainly early phase lesions with only slight fibrosis. No histological lesions of facial nerves.
H90	Aged mare	41 days	Yes	Marked advanced lesions of cauda equina. Early phase lesions of left trigeminal nerve histologically.
H140	8-yr old gelding	16 days	Yes	Advanced lesions of cauda equina. Marked early phase lesions in facial nerve ganglion.

Table 2 Binding of ^{125}I -bovine P_2 to horse sera

	Percentage binding of ^{125}I -bovine P_2				
	-ve antigen	Bovine P_2	Human P_2	Bovine BP	Human BP
Control (10)	5.0 $\pm$ 1.8	3.7 $\pm$ 1.0 (NS)	—	4.9 $\pm$ 1.7 (NS)	—
H17	10.2 $\pm$ 0.5 ($P < 0.01$)	4.6 $\pm$ 0.3 ($P < 0.01$)	5.5 $\pm$ 0.4 ($P < 0.01$)	9.3 $\pm$ 0.4 (NS)	9.6 $\pm$ 0.1 (NS)
H21	8.1 $\pm$ 0.8 ($P < 0.05$)	3.5 $\pm$ 0.1 ($P < 0.02$)	5.5 $\pm$ 1.1 (NS)	5.9 $\pm$ 0.6 (NS)	7.7 $\pm$ 0.4 (NS)
H90	65.0 $\pm$ 1.1 ($P < 0.001$)	5.6 $\pm$ 0.4 ($P < 0.001$)	9.7 $\pm$ 0.2 ($P < 0.001$)	60.2 $\pm$ 0.6 ($P < 0.05$)	63.3 $\pm$ 0.5 (NS)
H140	42.8 $\pm$ 1.3 ($P < 0.001$)	6.0 $\pm$ 0.9 ($P < 0.001$)	10.4 $\pm$ 0.2 ($P < 0.001$)	39.2 $\pm$ 1.5 ($P < 0.05$)	41.8 $\pm$ 1.8 (NS)

All sera were used at a final dilution of 1:10; the binding of ^{125}I - P_2 in the pellet is expressed as a percentage of the total counts added (10^4 c.p.m.), mean $\pm$ s.d. In the first vertical column (-ve antigen), only ^{125}I -bovine P_2 has been added to each of the sera and the percentage binding for H17, H21, H90 and H140 are each compared to the mean percentage binding obtained for the ten control horses. In all four other vertical columns, 50 μg of the stated unlabelled antigen were added in addition to the ^{125}I -bovine P_2 . In each horizontal row the percentage binding following incubation in the presence of 50 μg unlabelled antigen is compared with the percentage binding in the absence of unlabelled antigen (-ve antigen). NS, not significant.

decreases in control levels. A clearer picture of events is probably represented by the sera from H90 and H140 which bound 65.0% and 42.8% respectively of the added label in the absence of additional antigen. In these, 50 μg bovine P_2 inhibited the binding by 91.4% and 85.1% respectively and inhibition by 50 μg human P_2 was only slightly less. In contrast, 50 μg bovine central nervous system (CNS) basic protein inhibited only slightly (7.4% and 8.4% respectively) and 50 μg human CNS basic protein caused no inhibition. Titration of the NCE sera demonstrated increased binding of radiolabelled P_2 up to a dilution of 80 with H90 and H140 but only 20 with H17 and H21. This binding is compared in Fig. 1 with that of sera from four Lewis rats immunized with either 0.5 mg or 2.5 mg bovine peripheral myelin.

Sera from four horses with NCE bound significantly more radiolabelled bovine P_2 than did sera from clinically normal horses. In case H21, however, the observed binding was within 2 standard deviations of normal and it would therefore probably be more accurate to consider this value as a high normal. The elevated binding of radiolabelled P_2 observed in the other three cases of NCE was completely abolished by bovine P_2 and almost so by human P_2 . Human CNS basic protein had no effect whereas bovine CNS basic protein produced a slight but significant inhibition. The observed binding was therefore attributed to antibodies specific for P_2 . The appropriate homologous antigen (horse P_2) was not available for this study but the considerable species cross-reactivity observed for this protein previously⁴ and the pattern of inhibition produced by the added myelin proteins here leave no doubt about such a conclusion. In addition, neither histone nor lysozyme produced any inhibition (result not shown) and so the results are unlikely to be due to a charge effect. Considerable variation in the titre of horse anti- P_2 was observed. This variation does not correlate with the age or sex of the animals (see Table 1) or with the interval from onset of the disease to withdrawal of the blood sample. A possible explanation for this variation is that both H90 and H140 (which had relatively high titres of anti- P_2) had definite cranial nerve involvement, whereas there was no clinical evidence of this in H17 and H21. Also, the lesions of the cauda equina in H21 were less advanced compared with the other three cases (Table 1). It is therefore reasonable to postulate that H90 and H140 were affected with a more widespread and/or severe form of the disease than H17 and H21 and this is reflected in the levels of anti- P_2 . Reference to the anti- P_2 levels observed in our laboratory rats, however, reveals that even in controlled conditions of antigen exposure, age, sex and duration there is considerable variation in the humoral response (Fig. 1). The fact that two rats received five times the dose of antigen than the other two received is reflected neither in the observed neurological severity (grade 2 in all cases) nor in the ranking of antibody titres. Viewed in this light the variations in results with the horse sera may not need further explanation.

Whilst the presence of antibodies to P_2 in NCE may be an epiphenomenon caused by the release of myelin from inflamed nerves, such antibodies have not been observed in other naturally occurring neurites including various forms of peripheral neuropathy in man and tuberculoid and lepromatous leprosy (unpublished work). In addition, attempts to identify circulating antibodies to the CNS basic protein in multiple sclerosis in which there is considerable damage have yielded disappointing and conflicting results^{8,9}. Thus the unequivocal presence of antibodies to P_2 reported here raises the possibility that these circulating antibodies play a pathogenic role in NCE in the horse.

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Calcium-binding protein parvalbumin as a neuronal marker

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Ca^{2+} ions, involved in the control of many important physiological functions, require the presence of specific intracellular Ca^{2+} -binding proteins to exert their regulatory roles. Many of the latter have been extracted and purified. Thus, troponin-C regulates muscle contraction¹ and calmodulin is involved in a variety of fundamental cellular activities^{2,3}. Parvalbumin seems to be restricted to muscle and brain^{4–6} but its physiological role is not yet clear. In muscle it probably participates in a regulatory system operating mainly in fast contracting muscles^{7,8}, but in brain, even its anatomical distribution is unknown. We now describe the localization by immunohistological means of parvalbumin in neurones scattered throughout the central nervous system of the rat. Although the distributions of parvalbumin- and GABAergic neurones are similar, the physiological function of parvalbumin-immunoreactive neurones is not yet recognizable.

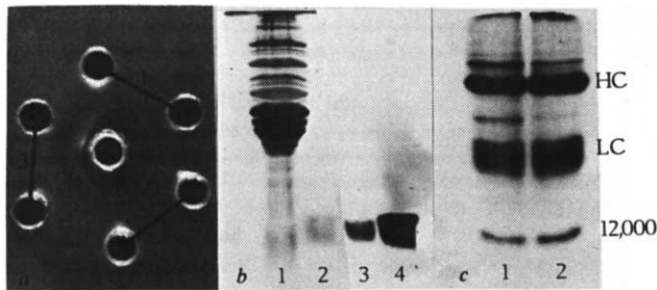


Fig. 1 Antibodies against homogeneous rat muscle parvalbumin were raised in rabbits as described elsewhere^{6,19}; the specificity was examined by four methods: *a*, double immunodiffusion of antiserum (centre well) against (1) total rat muscle extract, (2) muscle parvalbumin and (3) total brain extract. A single precipitin line was obtained. The same result was obtained by immunoelectrophoresis (not shown). *b*, Immunoreplica technique²⁰. SDS-polyacrylamide (15%) gels of (1) total muscle extract (100 µg protein) and (2) muscle parvalbumin (10 µg); (3) and (4) are the corresponding overlay gels (antiserum diluted 1:2 in 0.6% agarose). Proteins were stained with Coomassie blue. The antiserum reacted only at the site of the antigen. *c*, Immunoprecipitation. (1) Total muscle and (2) total brain extracts (4 mM EDTA, pH 7.5) were reacted with anti-muscle parvalbumin serum (2 h, 0 °C) in the presence of Triton X-100 and sodium deoxycholate (1% wt/vol each). After centrifugation, the immunoprecipitate was washed with phosphate-buffered saline (PBS) and finally ¹⁴C-labelled by reductive methylation²¹ using ¹⁴C-formaldehyde (specific activity 52 mCi mmol⁻¹, NEN). Labelled proteins were separated on a SDS-polyacrylamide (15%) gel and muscle and brain parvalbumins ($M_r = 12,000$) visualized by fluorography. HC, heavy chains; LC, light chains of the IgG antibody molecule.

For the immunohistochemical localization of parvalbumin, rat brains were perfusion-fixed with Bouin fluid and embedded in paraffin or treated otherwise (see Fig. 2*a* legend). Sections were mounted on slides and incubated with various dilutions of the parvalbumin antisera. The specificity of one of the two antisera used is given in Fig. 1. Sections were further processed according to the unlabelled antibody technique of Sternberger⁹. Control slides were processed simultaneously with preimmune sera of the same animals and with preadsorbed antisera.

The brain cells immunostained by the parvalbumin antisera were identified as neurones. Astro- and oligodendroglia, as well as the endothelia of blood vessels and smooth muscle cells, remained unstained. The immunostaining was always reproducible in the range of antisera dilutions used.

In all parvalbumin-positive neurones the soma, dendrites and axons were homogeneously labelled (Figs 2*a*, *b* and 4*d*) whereas nuclei were unreactive. Because our observations were restricted to the level of the light microscope, it was impossible to determine whether parvalbumin is bound to structural elements such as microfilaments or microtubules. Calmodulin, on the other hand, is associated with ribosomes, endoplasmic reticulum and plasma membranes^{10,11} and occurs in Purkinje and granular cells of the cerebellum¹⁰. Granular cells are not recognized by the parvalbumin antisera; thus the staining pattern is distinct and a cross-reaction of our antisera with calmodulin unlikely.

Parvalbumin-immunoreactive neurones were present in all areas of the neocortex, and in all layers except layer I (Fig. 3*a*). They were ~30 µm in diameter and in some areas were more numerous in layers II and IV. It was not possible to trace axons leaving the cortex and coursing in the cerebral peduncles; thus the parvalbumin-immunoreactive cells in the neocortex are probably not pyramidal cells.

In the olfactory bulb only periglomerular cells in the glomerular layer were recognized by parvalbumin antisera. In the hippocampus, single immunoreactive neurones within the pyramidal layer were observed, although no positive fibres could be found leaving with the fimbria hippocampi (Fig. 4*d*). The location, frequency and pattern of axon ramification of the immunoreactive neurones is suggestive of basket cells.

All Purkinje cells of the cerebellum showed strong parvalbumin immunoreactivity (Figs 2*b*, 4*a*). In the molecular layer, some basket and stellate neurones were labelled (Figs 2*b*, 4*a*), whereas the granular cell layer displayed no immunostaining. Both the dendritic trees and axons of Purkinje cells (Fig. 2*b*) were stained; the latter could be seen between unlabelled

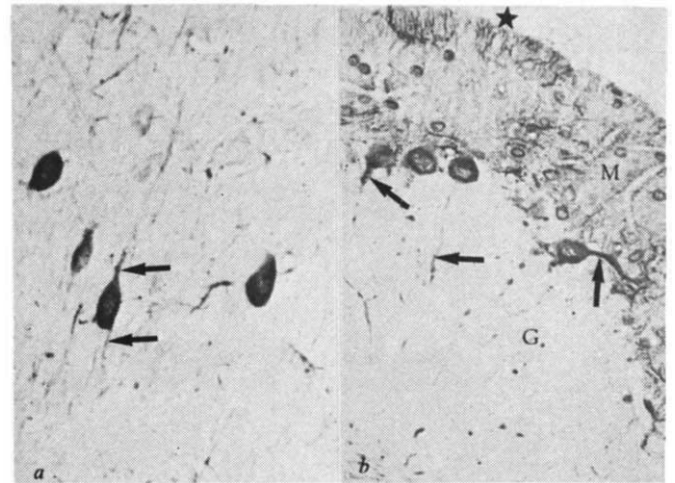


Fig. 2 *a*, High-power micrograph of a neuron in layer V showing unstained nuclei, heavily immunoreactive soma and cell processes (arrows). Corresponds to inset of Fig. 3*a*. $\times 335$. For this and the subsequent figures, the brains of 10 albino rats of two different strains (Wistar and Sprague-Dawley) and of both sexes were fixed by intra-aortic perfusion with 500 ml of Bouin fluid, dehydrated in graded ethanol and embedded in paraplast. 5 µm-thick sections were mounted on chromalaun-gelatine-coated slides and incubated for at least 48 h in a moist chamber at 4 °C. The parvalbumin antisera were used in a dilution of 1:1,000 until 1:10,000 in PBS, dilutions shown in pilot experiments to give the best staining background ratio. After thorough washing, the sections were exposed for 30 min at room temperature to goat anti-rabbit IgG (Nordic; 1:30 in PBS) and, after a further washing step, to peroxidase-antiperoxidase complex (PAP; Sternberger-Meyer Immunocytochemical, Maryland; 1:100 in PBS). The peroxidase activity was demonstrated histochemically with diaminobenzidine/H₂O₂ (0.04%:0.004% for 5 min). Sections were dehydrated and mounted. Alternatively, rat brains were perfusion-fixed with 500 ml of 10% paraformaldehyde in cacodylate buffer pH 7.4, 0.1 M for 30 min and then cut to 50 µm-thick sections in a vibratome (Oxford). Floating sections were incubated overnight and processed identically as described for paraffin sections. *b*, Sagittal section through a cerebellar folium displaying intense immunoreactivity of perikarya, axons (arrow), main dendritic branches (arrow head) and dendritic trees (asterisk) of all Purkinje cells. Basket and stellate cells in the molecular layer (M) are labelled. Note that the parvalbumin antiserum stained Purkinje cells at a much higher dilution (up to 1:20,000) than neurones in the rest of the brain, indicating much higher density of antigenic sites in Purkinje cells. G, granular layer without immunoreaction.

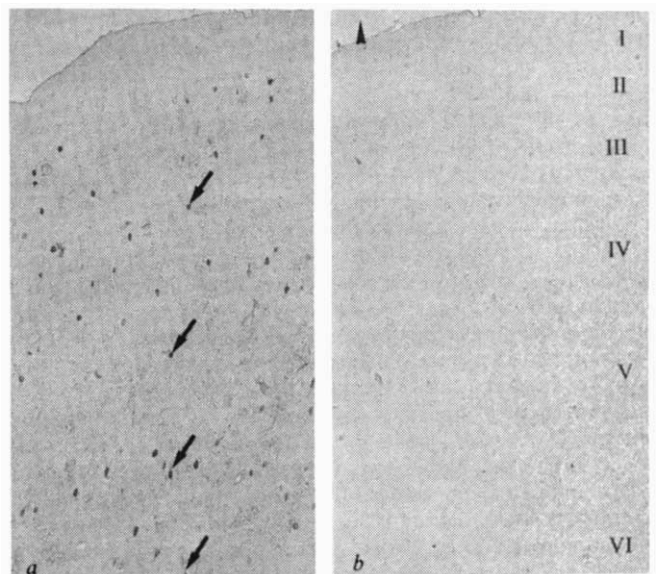


Fig. 3 Low-power micrographs of two consecutive serial sections through the rat cerebral cortex (area praecentralis 4-6 in ref. 22) incubated with a parvalbumin antiserum (*a*) and preimmune serum (*b*). The parvalbumin antiserum stains neurones (some of which are marked by an arrow) scattered over various layers (II-VI) of the cerebral cortex. A meshwork of cell processes running in various directions is distinguishable particularly in the deep layers. The preimmune serum (*b*) does not stain any structures in the brain. Identical pictures were obtained after adsorbing 100 µl of the undiluted parvalbumin antisera with 10⁻⁹ M of the antigen for 48 h at 4 °C. $\times 96$.

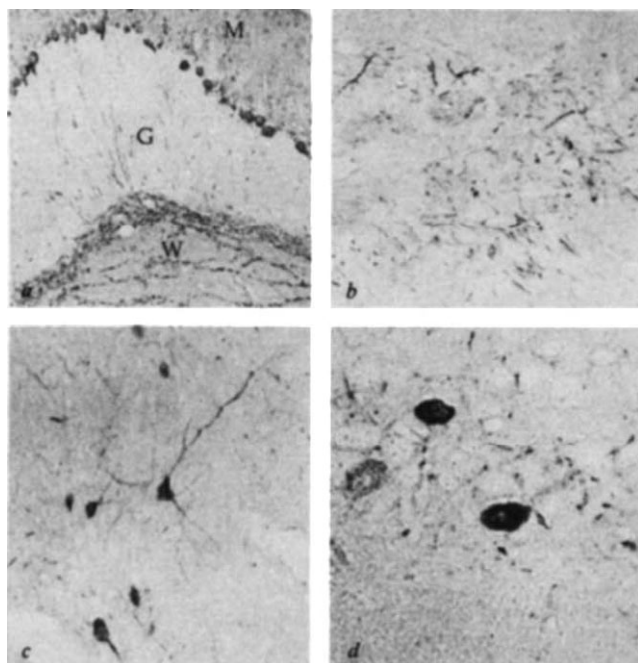


Fig. 4 *a*, Cerebellum with molecular (M) and granular layers (G) which sandwich the Purkinje cells layer. The Purkinje cell axons run in the granular layer (G) and form bundles in the white matter (W). $\times 74$. *b*, Sagittal section of anterior part of the ventral nucleus of the thalamus (after ref. 23). Intermingled cell processes, but not cell bodies, are recognized by the parvalbumin antiserum. $\times 155$. *c*, Hypothalamus, lateral preoptic area (after ref. 23). A large parvalbumin-immunoreactive neurone sends processes in all directions. $\times 155$. *d*, Hippocampus (CA₁ region). A few parvalbumin-immunoreactive cells and fibres are scattered between unlabelled neurones of the pyramidal-cell layer. $\times 350$.

granular cells in the white matter (Fig. 4*a*) and making contact with neurones in the deep cerebellar nuclei (not shown).

In the hypothalamus immunoreactive neurones were widely scattered except for some clustering in the lateral preoptic area (Fig. 4*c*) and in the premammillary nuclei. In contrast to other regions, in the specific thalamic nuclei nerve fibres, but no cell bodies, were labelled (Fig. 4*b*). In contrast, the perikarya of the nucleus reticularis thalami were heavily stained (not shown). Very small immunoreactive neurones and positive punctate structures could be seen in Rexed lamina IIb of the dorsal horn of the spinal cord. Labelled terminals surrounded motoneurones in the ventral horn and heavily immunoreactive myelinated axons coursed in the dorsal funiculi (not shown). In spinal ganglia immunolabelling was restricted to large cells and in peripheral nerves to large-diameter axons (not shown).

The selective staining of neurones by parvalbumin antisera indicates that this protein is a marker of a distinct neuronal population. Other neurone-specific antigens are the neurotransmitter synthesizing enzymes, olfactory marker protein, γ - γ -enolase and some neurone-specific surface antigens (for recent reviews see ref. 12). Some of these antigens occur only in local subgroups of neurones segregated in certain brain areas; the function of most of them is unknown. On the other hand, parvalbumin is a highly specific calcium-binding protein in muscle and could function similarly in the brain. Although we cannot yet classify parvalbumin-positive neurones as members of a homogeneous functional group, Ca²⁺ ions may have a peculiar role in the physiology of these cells. Parvalbumin might be a marker for neurones displaying Ca²⁺ action potentials¹³, as is the case for Purkinje cells¹⁴, although other Ca²⁺ spike-producing cells (small neurones in spinal ganglia¹⁵) are not recognized by the parvalbumin antisera.

The Ca²⁺-binding protein parvalbumin in the central and peripheral nervous system could be functionally related to axonal flow, which is known to be Ca²⁺ dependent¹⁶. Axoplasmic transport studies are always performed on whole nerve preparations and the resultant flow rate is the sum of the rates in

individual nerve fibres. Study of axoplasmic transport in single-fibre preparations may show whether parvalbumin-immunoreactive axons display distinct axoplasmic transport rates.

Similarities exist between the distribution of parvalbumin-positive neurones and those containing the inhibitory neurotransmitter γ -aminobutyric acid (GABA). The parvalbumin-positive Purkinje, basket and stellate cells in the cerebellum, the basket cells in the hippocampus and periglomerular cells in the olfactory bulb all use GABA as neurotransmitter (for review see ref. 17). However, the confirmation of a preferential association of parvalbumin with GABA-ergic neurones awaits further studies. A cross-reaction of the parvalbumin antiserum with GABA-synthesizing enzymes is unlikely; the molecular weight of the brain protein recognized by the parvalbumin antiserum (12,000, Fig. 1) is in fact much lower than the molecular weight of the GABA-synthesizing enzymes¹⁸.

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Cytochalasin D inhibits actin polymerization and induces depolymerization of actin filaments formed during platelet shape change

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Cytochalasins, a class of fungal metabolites, affect a wide variety of motile functions of eukaryotic cells¹⁻³. Recently, several laboratories have shown that cytochalasins inhibit actin polymerization *in vitro*, presumably by binding with high affinity to growing ends of actin nuclei and filaments (F-actin), and preventing addition of monomers (G-actin) to these sites⁴⁻⁸. Cytochalasins have also been reported, in certain conditions, to induce depolymerization of actin filaments *in vitro*⁶. However, correlations of these effects of cytochalasins on actin polymerization *in vivo* have been limited to electron microscopic studies. Recently, Morris and Tannenbaum reported that there was no net depolymerization of actin in spreading fibroblasts treated with cytochalasin D, despite the well known morphological effects of the drug on these cells⁹. Here, we describe results indicating that cytochalasin D can inhibit the rapid polymerization of actin in human platelets after thrombin stimulation and induce rapid depolymerization of filamentous actin in stimulated platelets. Both of these effects correlate with observed changes in platelet shape.

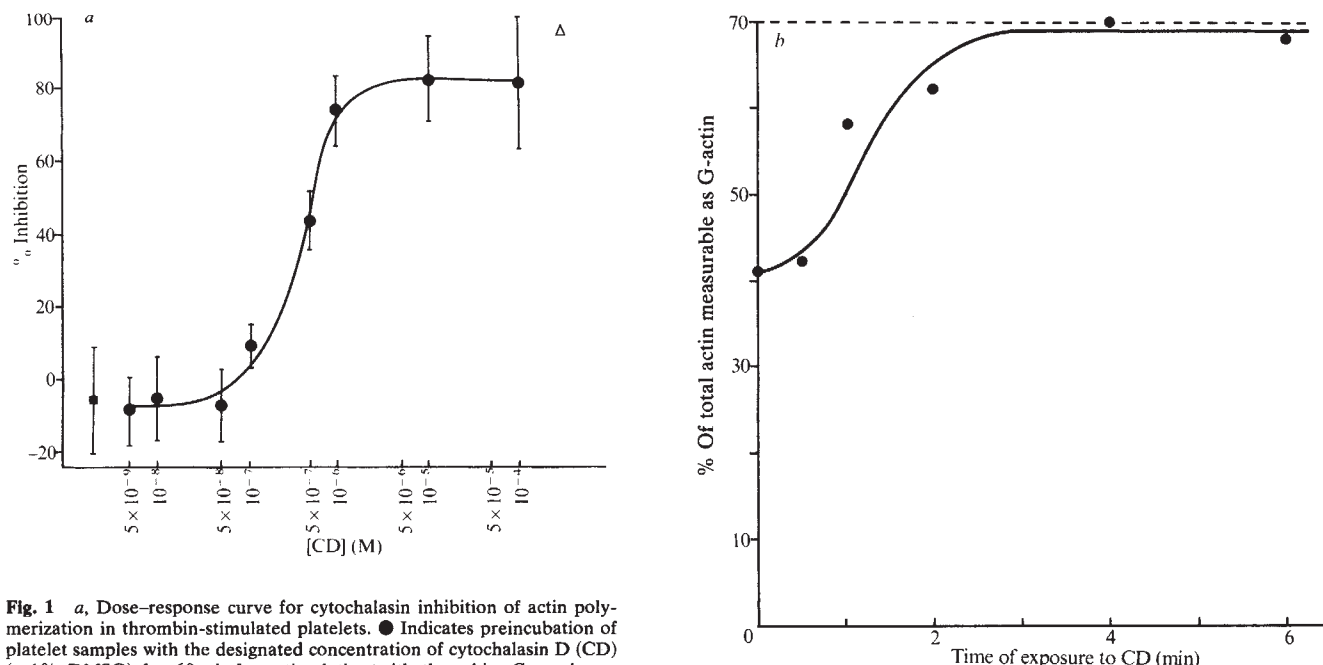


Fig. 1 *a*, Dose-response curve for cytochalasin inhibition of actin polymerization in thrombin-stimulated platelets. ● Indicates preincubation of platelet samples with the designated concentration of cytochalasin D (CD) (+1% DMSO) for 60 s before stimulation with thrombin. Controls are indicated by: △, DMSO (final concentration 1%) added to unstimulated platelets for 60 s; ■, DMSO (final concentration 1%) added to platelets 60 s before stimulation with thrombin. Per cent inhibition is defined by the equation: % Inhibition = $\{([G\text{-actin}]_{\text{sample}} - [G\text{-actin}]_{\text{stim}}) / ([G\text{-actin}]_{\text{unstim}} - [G\text{-actin}]_{\text{stim}})\} \times 100$. Error bars indicate s.e.m. for two to six samples. *b*, Time dependence of the increase in G-actin concentration of stimulated platelets after exposure to cytochalasin D. Platelets were stimulated with thrombin and then exposed to 10^{-6} M cytochalasin D for the time indicated before lysis. The dotted line indicates G-actin values for unstimulated platelets in this experiment. In both *a* and *b*, as well as experiments described in the text, fresh platelets were obtained from venous blood of healthy human donors who had not ingested aspirin or other platelet-inhibitory drugs for a minimum of 10 days. Blood was collected in plastic syringes containing acid citrate dextrose in the ratio of 1 part 0.085 M trisodium citrate, 0.065 M citric acid, 2% dextrose to 5.25 parts of whole blood, to achieve a final pH of 6.45–6.50. Platelets were then separated from other cellular elements by differential centrifugation and concentrated by a single centrifugation at 1,000g, followed by resuspension in one-quarter volume of the resulting platelet-poor plasma. The platelets were then separated from plasma constituents by passage through a Sepharose 2B column¹⁸ (Pharmacia) and eluted in phosphate-buffered saline (PBS) containing 137 mM NaCl, 5 mM KCl, 1 mM KH_2PO_4 , 1 mM Na_2HPO_4 , 0.6 mM MgSO_4 , 0.05 mM CaCl_2 , 8 mM glucose and 16 mg ml^{-1} bovine serum albumin (Sigma), pH 6.9. EDTA (final concentration 1 mM) was added to platelet samples immediately before use and the pH was adjusted to 7.4. Platelets were lysed after the experimental manipulations as indicated by addition of 0.1 volume of 10% Triton X-100, 20 mM MgCl_2 , 20 mM EGTa, 2 mM ATP and 5 mM dithiothreitol, pH 7.4. G-actin was then assayed ~45 s after lysis by the DNase inhibition assay as described by Blikstad *et al.*¹⁰. Total actin concentrations were determined after 1:1 dilution of platelet suspensions with a depolymerization buffer containing 1.5 M guanidine HCl, 1 M sodium acetate, 1 mM CaCl_2 , 1 mM ATP, 20 mM Tris-HCl, pH 7.5, and incubation for at least 5 min at 4 °C, with frequent vigorous agitation. Platelets were stimulated by addition of 1 NIH unit ml^{-1} of human thrombin (Sigma) and constant agitation for 90 s. (DNA and DNase I were from Worthington.)

The G-actin content of platelets was measured in a variety of conditions using the DNase enzyme inhibition assay of Blikstad *et al.*¹⁰. Thrombin stimulation of platelets in the presence of sufficient EDTA to prevent aggregation resulted in a marked reduction in G-actin concentrations from a mean of $67.5 \pm 1.4\%$ (s.e.m., $n = 32$) of total cellular actin in lysates of unstimulated platelets to a mean of $36.8 \pm 1.2\%$ ($n = 25$) in lysates of stimulated cells. These results are similar to those observed by Carlsson *et al.*¹¹, who compared unstimulated platelets with platelets aggregated by thrombin in the presence of calcium. The addition of 10^{-6} M cytochalasin D 1 min before thrombin stimulation almost completely obliterated this response (G-actin level = $62.4 \pm 2.1\%$, $n = 21$). Moreover, the addition of cytochalasin D to platelets which had previously been stimulated with thrombin resulted in a return of G-actin levels at 3 min to those seen in unstimulated platelets ($69.4 \pm 2.0\%$, $n = 8$). The addition of cytochalasin D to lysates of stimulated platelets immediately after lysis did not significantly affect G-actin levels ($38.4 \pm 2.4\%$, $n = 5$), thus indicating that the effect of cytochalasin D on stimulated platelets is due to treatment of intact cells, and is not secondary to an effect of the drug on actin filaments after cell lysis. Cytochalasin D treatment of unstimulated platelets for 1 min did not affect G-actin levels ($66.4 \pm 2.2\%$, $n = 5$); similar results were obtained with longer incubation periods. Dimethyl sulfoxide (DMSO), the carrier for cytochalasin D, did not significantly affect stimulated or unstimulated platelets in the concentration (1%) used.

Figure 1a shows a dose-response curve for cytochalasin D inhibition of thrombin-stimulated actin polymerization. Half-

maximal inhibition was reached at $\sim 5 \times 10^{-7}$ M, similar to that required for 50% inhibition of nuclei-induced actin polymerization *in vitro*⁵. The cytochalasin D reversal of thrombin-induced actin polymerization was further investigated by timed exposure of thrombin-stimulated platelets to the drug. As Fig. 1b shows, the amount of G-actin present increased time-dependently until it reached the baseline level for unstimulated platelets.

Electrophoretic analysis of the actin content of Triton-insoluble residues obtained by low speed centrifugation of the platelet lysates¹² confirmed our results from the DNase assay. Lysates of thrombin-stimulated platelets showed a dramatic increase in the amount of actin associated with the Triton residue, which was inhibited by preincubation with cytochalasin D (Fig. 2). The addition of cytochalasin D after thrombin stimulation also resulted in a decrease in the amount of actin in the Triton residue. The amount of actin recovered in parallel experiments using centrifugal forces high enough to pellet individual actin filaments again approximated the amount recovered from unstimulated platelets in the same conditions (data not shown). Thus, it is unlikely that the effects of cytochalasin D were due to interference with attachment of actin filaments to larger cytoskeletal structures. Figure 2 also shows that a band co-migrating with tubulin (50–55,000 molecular weight doublet) increased dramatically in stimulated platelets, and this increase was not affected by cytochalasin D. Therefore, although unstimulated and cytochalasin-treated stimulated platelets are similar in their G- and F-actin content, they would not be expected to be structurally identical.

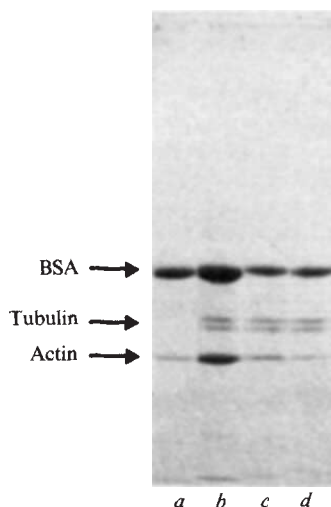


Fig. 2 Quantitative SDS-polyacrylamide gel electrophoresis of Triton residues of unstimulated platelets (a), thrombin-stimulated platelets (b), platelets preincubated with cytochalasin D and then stimulated with thrombin (c), and platelets stimulated with thrombin and then incubated with cytochalasin D (d). These experiments were performed according to the protocol outlined in the text and Fig. 1 legend, except that the platelet extracts were centrifuged at 17,800g for 4 min immediately after Triton lysis. The pellets were then washed once (without resuspension) in a solution of 1 part lysis buffer to 10 parts of the platelet suspension buffer described in Fig. 1 legend and recentrifuged at the same speed. The pellets were stored frozen at -70°C for 12–72 h. In preparation for quantitative analysis on SDS-polyacrylamide slab gels, samples were solubilized in a 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.0625 M Tris-HCl solution, pH 6.8, and incubated at 56°C for 2 h, with frequent vortexing and sonication. Samples were then electrophoresed through 3% acrylamide stacking gels and 7% acrylamide separating gels according to the method of Laemmli¹⁹. The gels were stained for protein with Coomassie brilliant blue. Actin and tubulin bands were identified by comparison with actin and tubulin standards. The BSA band represents bovine serum albumin added to the platelet suspension buffer. Each lane contained Triton residue derived from equal numbers of platelets. This allows relative amounts of sedimentable actin to be compared from gel to gel for the experimental conditions indicated.

Studies were also performed using scanning electron microscopy to compare the morphology of platelets (Fig. 3). With the preparative procedures described, 80–90% of the platelets maintained a diskoid form. Many of these diskoid forms also had one or more spicules, or pseudopods. In contrast, after thrombin stimulation, diskoid forms were completely absent, the number of pseudopods on each platelet increased and the platelet surface developed a convoluted or 'folded' appearance. With cytochalasin D treatment before or after stimulation, fewer pseudopods were seen and the surface of the platelet appeared much less convoluted. None of the platelets maintained their original diskoid form.

Our results indicate that cytochalasin D is capable of inhibiting the rapid polymerization of actin *in vivo* and inducing depolymerization of actin in stimulated platelets, both resulting in identical changes in platelet surface morphology. Although other investigators have postulated the disruption of actin filaments by cytochalasins in other cell types based on morphological criteria³, there has been no previous convincing biochemical demonstration of either inhibition of actin polymerization or depolymerization of actin by cytochalasins in a cellular system. The finding that the conversion from diskoid to non-diskoid shape was not affected by cytochalasin D is consistent with previous studies suggesting that loss of diskoid form is a microtubule-related function^{2,13}. This notion is also supported by our results indicating that the amount of tubulin in the Triton-insoluble residues was dramatically different in unstimulated and stimulated platelets, and did not seem to be affected by cytochalasin D.

It is interesting that cytochalasin D treatment of stimulated platelets resulted in a return of G-actin levels to that seen in unstimulated platelets, whereas cytochalasin D treatment of unstimulated platelets resulted in no comparable change in G-actin levels. We suspect that this represents a stabilized structural pool of F-actin (not depolymerizable by cytochalasin D) with markedly different properties from the additional F-actin present immediately after rapid polymerization occurs, such as actin filaments complexed with tropomyosin-troponin¹⁴, or stabilized actin nuclei that may be involved in the control of polymerization of actin in platelets^{15,16}. The well known morphological effects of cytochalasins on spreading fibroblasts

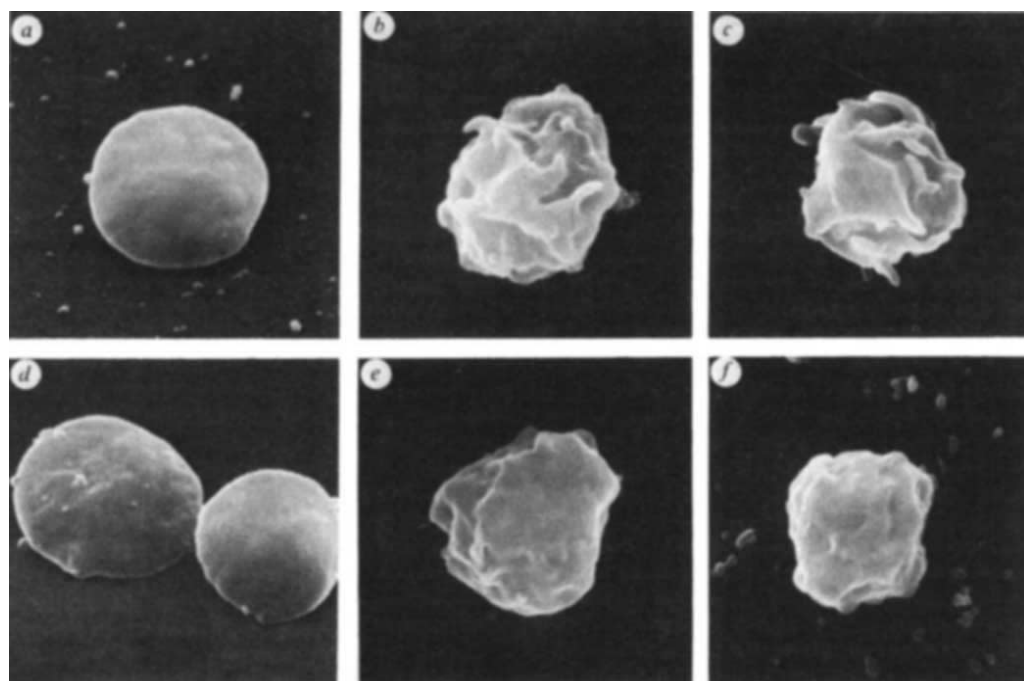


Fig. 3 Scanning electron micrographs showing the representative morphology for unstimulated platelets (a), stimulated platelets (b, c), unstimulated platelets incubated with cytochalasin D (d), platelets preincubated with cytochalasin D and then stimulated with thrombin (e), and platelets stimulated with thrombin and then incubated with cytochalasin D (f). These experiments were performed in conditions identical to those described in Figs 1 and 2 legends, except that in lieu of lysis the platelet suspension was diluted 1:10 with a suspension buffer consisting of the PBS previously described (Fig. 1 legend) with 4% added glutaraldehyde and the bovine serum albumin omitted. After fixation for 5 min at room temperature, the platelets were allowed to settle for 20 min on coverslips previously coated with poly-L-lysine (Sigma). The coverslips were then placed in a larger volume of fresh glutaraldehyde-containing buffer for 1–2 h and transferred to PBS without albumin for overnight storage. The platelet-containing coverslips were prepared for scanning electron microscopy by serial dehydration in alcohol, critical point drying in a Polaron critical point apparatus with liquid CO_2 as the transition fluid, and coating with gold-palladium in a Polaron sputter coater (model E5100 series II). They were then examined in a JEOL JSM-35 scanning electron microscope at accelerating voltages of 18 kV. All photographs are at magnifications of $1.8 \times 10^4\times$.

(such as HEP-2 cells) may well be the result of cytochalasin effects on actin filament-filament interaction^{8,17}, and such changes might not be reflected in a change in G-actin concentrations detectable by the DNase assay. Therefore, our results and those of Morris and Tannenbaum are not necessarily contradictory and support the notion that there are two types of cytochalasin-sensitive motile processes, the first involving actin polymerization and depolymerization, the second involving changes in filament-filament interactions.

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Increased phosphotyrosine content and inhibition of proliferation in EGF-treated A431 cells

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Epidermal growth factor (EGF), which binds to specific high-affinity cell-surface receptors, stimulates replication of a number of cell types^{1,2}. *In vitro* EGF stimulates a membrane-associated protein kinase which catalyses phosphorylation of the EGF receptor at tyrosine residues^{3,4}. The transforming proteins of several RNA tumour viruses are protein kinases which also specifically catalyse phosphorylation at tyrosine residues⁵⁻¹⁰. An elevated level of phosphotyrosine is found in cells transformed by Rous sarcoma, Fujinami sarcoma, PRCII, Y73, Snyder-Theilin and Gardner-Armstrong feline sarcoma and Abelson murine leukaemia viruses^{5,10-12}. At least four of these viruses, which encode distinct protein kinases, catalyse phosphorylation of tyrosine residues in the same cellular substrate proteins¹³. *In vitro* EGF-stimulated protein kinase catalyses the phosphorylation of anti-p60^{src} heavy chains, suggesting that this enzyme recognizes similar substrate determinants to p60^{src} (refs 14, 15). Here we demonstrate that EGF treatment of A431 human epidermoid carcinoma cells increases phosphotyrosine content, indicating that EGF stimulates tyrosine-specific protein kinase activity *in vivo* as well as *in vitro*. In contrast to Rous sarcoma virus (RSV) transformation, EGF inhibits replication of A431 cells. This inhibition by EGF is influenced by both cell density and tissue culture substratum.

Although phosphotyrosine is a rare phosphoamino acid, transformation of chick embryo fibroblasts with RSV results in an increase in phosphotyrosine content from 0.03 to 0.3% of cellular phosphoamino acids⁵. Analysis of transformation using RSV containing a temperature-sensitive mutation in the trans-

forming protein p60^{src} indicates that the increase in cellular phosphotyrosine content results from p60^{src} kinase activity¹¹. Because EGF also stimulates tyrosine-specific protein kinase activity, the effect of EGF on phosphotyrosine content of exponentially growing A431 cells was determined. After the cells had been labelled to equilibrium with ³²P-orthophosphate, replicate plates were treated with EGF for 3 h and phosphotyrosine content was measured. As shown in Fig. 1, EGF increased the phosphotyrosine content of A431 cells sevenfold (0.14 and 0.02% in EGF-treated and untreated A431 cells respectively), indicating that EGF activates tyrosine-specific protein kinase activity *in vivo*. The average increase in phosphotyrosine content in EGF-treated A431 cells was 5.4-fold in four separate experiments. This result suggests that the EGF-stimulated protein kinase activity observed *in vitro*³ is an important component of the mechanism of EGF action.

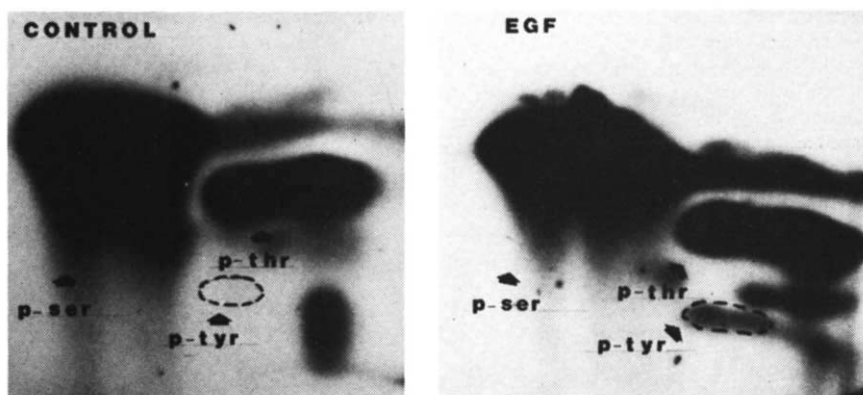
A431 human epidermoid carcinoma cells have an unusually high density of EGF receptors^{16,17}. In response to EGF, A431 cells undergo a rapid shape change with formation of ruffles and filopodia¹⁸, and exhibit rounding and extension of long processes, a phenomenon enhanced by lowering the Ca²⁺ concentration of the medium¹⁹. EGF also induces clustering of EGF receptors¹⁷ and stimulates the rate of fluid pinocytosis²⁰. Although EGF stimulates replication of many different cell types, it was found to inhibit replication of A431 cells. As shown in Fig. 2, EGF inhibited replication of low-density A431 cells growing on tissue culture dishes at a half-maximally effective concentration (EC₅₀) of ~0.1 nM. At higher densities, higher concentrations of EGF were required for growth inhibition. At an initial cell density of 11,000 cells cm⁻², the EC₅₀ for EGF inhibition of cell growth was ~1 nM. After addition of saturating concentrations of EGF, A431 cells underwent approximately one cell doubling; growth inhibition was then complete at both low and high cell densities. The growth inhibitory effects of EGF were sustained. There was no growth when A431 cells were maintained with saturating concentrations of EGF over a 14-day period.

To evaluate the possibility that an alteration in EGF receptor affinity or concentration was responsible for the effect of cell density on the response to EGF, EGF receptors were measured in A431 cells growing at densities varying from 600 to 49,000 cells cm⁻². As shown in Fig. 3, there is an inverse relationship between cell density and the affinity of EGF receptors for EGF. The equilibrium dissociation constant, K_D, increased from 0.7 nM at 600 cells cm⁻² to 11 nM at 28,000 cells cm⁻² and did not seem to increase further at higher cell densities. EGF receptor number also varied with cell density, increasing from 0.5 pmol ¹²⁵I-EGF bound per 10⁶ cells at low cell density to 5.7 pmol ¹²⁵I-EGF bound per 10⁶ cells at high cell density (49,000 cells per cm²).

The measured K_D for EGF binding at both low and high cell densities was higher than the EC₅₀ of EGF required to inhibit cell proliferation at that cell density (sevenfold at 500 cells cm⁻² and fourfold at 15,000 cells cm⁻²), indicating that occupancy of only a fraction of EGF receptors is required for growth inhibition. The EC₅₀ for growth inhibition by EGF increased as expected when the affinity of receptors for EGF decreased. However, the number of EGF receptors increased. Increased receptors would tend to enhance sensitivity at higher cell densities^{21,22} and thus have an effect on the dose-response curve for the biological effect opposite to that of the measured decrease in EGF receptor affinity. Because density-dependent changes in EGF receptors did not fully explain the altered dose-response curves for EGF-mediated inhibition of cell proliferation, additional factors were considered.

As the tissue culture substratum is an important determinant of cell proliferation²³, A431 cells grown on coverslips were compared with those grown on tissue culture dishes. As shown in Fig. 2, EGF inhibited the growth of high-density (15,000 cells cm⁻²) A431 cells grown on the coverslip substratum and of low-density A431 cells (1,000 cells cm⁻²) with respective EC₅₀s of 0.2 and 0.01 nM. This is approximately 10-fold less EGF than

Fig. 1 Effect of EGF on phosphotyrosine content of A431 cells. Exponentially growing A431 cells were placed in 2 ml of phosphate-free Dulbecco's modified Eagle's medium (DMEM) containing 5% dialysed calf serum and 5% undialysed fetal calf serum. ^{32}P -orthophosphate (1 mCi ml^{-1}) was added and cells were labelled to equilibrium (18 h). EGF ($0.1\text{ }\mu\text{M}$) was then added to one group of dishes for 3 h. Cells were rapidly washed with cold phosphate-buffered saline (PBS) and removed by addition of RIPA buffer³⁵. Protein was extracted with phenol, precipitated with trichloroacetic acid and the pellet washed with chloroform/methanol (2:1)¹¹. The dried pellet was dissolved in 6 M HCl and hydrolysed at 110°C for 2 h. After lyophilization and resuspension, authentic phosphoserine, phosphothreonine and phosphotyrosine standards were added and phosphoamino acids were separated by electrophoresis on $100\text{-}\mu\text{m}$ cellulose thin layer plates. Approximately 2.5×10^6 c.p.m. were added to each plate. Electrophoresis was at pH 1.9 in the first dimension (2 kV for 1.5 h) and at pH 3.5 in the second dimension (1 kV for 1 h)¹¹. Autoradiographs were made using pre-flashed film; to visualize phosphotyrosine, plates were overexposed for phosphoserine and phosphothreonine. Phosphoamino acids were then carefully scraped from the plate and counted. Phosphotyrosine content was calculated as a percentage of total phosphoamino acids present. Control cell exposure time was 18 h, that for EGF-treated cells was 17 h. Control cells contained 94.6% phosphoserine, 5.38% phosphothreonine and 0.02% phosphotyrosine. After subtraction of background, there were 146 c.p.m. in phosphotyrosine. EGF-treated cells contained 94.93% phosphoserine, 4.93% phosphothreonine and 0.14% phosphotyrosine. After subtraction of background, there were 803 c.p.m. in phosphotyrosine.



required when cells were grown at similar densities on tissue culture dishes. As shown in Fig. 3, growth on coverslips affected neither the affinity of receptors for EGF nor the number of EGF receptors per cell ($K_D = 0.57$ and 4.9 nM , and maximum amount bound = 1.0 and $4.8\text{ pmol EGF bound per } 10^6$ cells at cell densities of 900 and $15,000\text{ cells cm}^{-2}$ respectively). Cells grown on coverslip substratum exhibited differences between the K_D for EGF binding and the concentration required for half-maximal inhibition of cell growth which exceeded those exhibited by high-density cells grown on tissue culture substratum. Although the number of EGF receptors and the affinity of the receptors for EGF were similar at each cell density for cells grown on dishes or coverslips, the dose-response curve for EGF for the biological response of growth inhibition was strongly affected by the tissue culture substratum.

The possibility that increased EGF degradation at high cell densities on tissue culture dishes contributed to the rightward shift in the dose-response curve shown in Fig. 2 was evaluated by increasing the medium/cell ratio sixfold so that at any concentration of EGF the total amount present in the culture medium was increased. In these conditions the EC_{50} for EGF inhibition of cell proliferation remained constant (data not

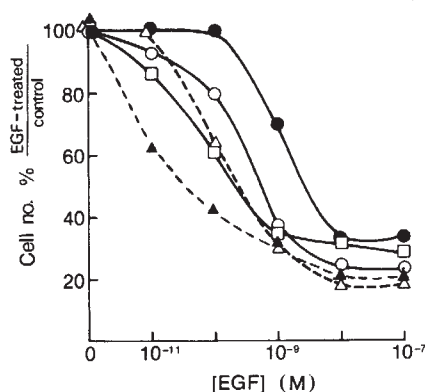


Fig. 2 Effect of EGF on growth of A431 cells plated at different cell densities. A431 cells were subcultured into 3-cm plastic tissue culture dishes (Falcon) at 550 ($\square$), $2,500$ ($\circ$) and $11,000$ ($\bullet$) cells cm^{-2} . After attachment overnight, medium was replenished with 2 ml fresh DMEM containing 10% calf serum and the indicated concentrations of EGF. Medium with the same concentration of EGF was replenished every 2 days and triplicate dishes of cells were counted on day 6. In the absence of EGF, cells underwent four population doublings. Cells were also seeded on to 2.5-cm^2 coverslips (Thermanox, Lux Scientific Corporation) at a density of $1,000$ ($\blacktriangle$) and $15,000$ ($\triangle$) cells cm^{-2} . After attachment of cells, coverslips were placed with a drop of agar as anchor, into 6-cm dishes containing DMEM, 10% calf serum and the indicated concentrations of EGF. All points are means of triplicate dishes or coverslips which differed by $<5\%$.

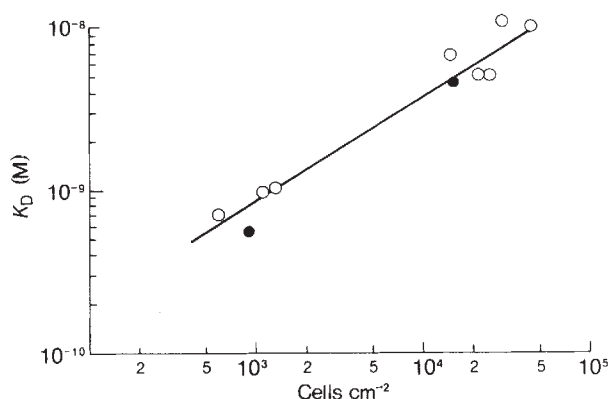


Fig. 3 Effect of cell density on binding of EGF to A431 cells. A431 cells were subcultured into 3-cm tissue culture dishes and used 24–48 h later. Cells at the indicated densities were washed three times with PBS, then incubated for 1 h (equilibrium) at 25°C with PBS containing 0.1% bovine serum albumin (BSA), $0.23\text{ nM } ^{125}\text{I}$ -EGF ($154\text{ }\mu\text{Ci per }\mu\text{g}$) and increasing concentrations of unlabelled EGF. At the end of the incubation, cells were washed three times with PBS/0.1% BSA, removed with $0.1\text{ M NaOH}/10\%$ SDS and radioactivity was determined. Duplicate assays were carried out at each EGF concentration. Data were plotted by the method of Scatchard and K_D was determined by linear regression analysis using a computer program. All lines gave $r^2 > 0.88$ and exhibited a single class of affinity sites. ^{125}I -EGF binding measured on cells grown at low density was confirmed using increasing amounts of EGF of constant specific activity. $\circ$, EGF binding to A431 cells grown on tissue culture dishes; $\bullet$, binding to cells grown on coverslips. Cultures were devoid of mycoplasma contamination as determined by autoradiography and ^3H -uracil uptake³⁶.

shown), suggesting that altered degradation of EGF does not contribute to the effect of cell density on the growth response of A431 cells to EGF. Changes in medium/cell ratios also failed to alter the EGF dose-response curves for cells grown on coverslips. There was no evidence of down-regulation of EGF receptors in the presence of EGF.

Several conditions have been reported to affect EGF receptors. In BSC1 cells, the number of EGF receptors per cell decreased at high cell density²⁴, whereas the number of receptors per cell increased at high density in BALB/c-3T3 cells²⁵. Retinoids have also been reported to enhance the number of EGF receptors per cell²⁶. Phorbol esters decreased the affinity of receptors for EGF in direct proportion to their activity as tumour promoters²⁷; saccharin and cyclamate also decreased EGF receptor binding, presumably through decreased affinity²⁸. In A431 cells, density strongly affected EGF receptors. Increasing cell density 10-fold produced 10-fold increases in both the number of receptors per cell, from 3×10^5 to 3×10^6 per cell, and

the K_D , from ~ 1 to 10 nM. The effects of cell density on EGF receptors were independent of the tissue culture substratum, but the biological response to EGF was strongly dependent on this. On both substrata used, EGF inhibited cell proliferation, but the translation of EGF receptor occupancy into the biological response was more efficient on coverslips than on tissue culture dishes. The environmental factors of cell density and cellular attachment thus both significantly affect EGF action.

Several retrovirally transformed cells show a close correlation between the tyrosine-specific protein kinase activity of the transforming protein, increased phosphotyrosine content and cell proliferation¹³. The present studies point to a dissociation between phosphorylation of cellular proteins at tyrosine residues and stimulation of cell proliferation. EGF, which is mitogenic for many cell types *in vivo* and *in vitro*, stimulates tyrosine-specific protein kinase activity in membranes prepared from A431 (refs 3, 4), placenta²⁹ and other cell types³⁰. EGF also increases phosphotyrosine content of A431 cells and, as reported by Hunter and Cooper³¹, increases tyrosine phosphate in a 39,000-molecular weight protein in A431 cells. This protein is a substrate for p60^{src} and other retroviral transforming proteins *in vivo* and *in vitro*^{13,32,33}. However, EGF is inhibitory to A431 cell proliferation at concentrations equivalent to those which are stimulatory for proliferation in other cell types^{1,2}. A recent report indicates that EGF also inhibits growth of GH₄C₁ cells³⁴. If EGF action is mediated via tyrosine phosphorylation, the effects on cell growth will clearly depend on other processes within specific cells. It is also apparent that an increase in total cell protein phosphotyrosine content and in specific proteins such as the 39,000-molecular weight protein does not reflect the growth state of the cell. In certain conditions, phosphorylation of tyrosine residues may be associated with inhibition as well as with stimulation of cell proliferation.

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Precursor forms of penicillin-binding proteins 5 and 6 of *E. coli* cytoplasmic membrane

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Most secreted proteins of both eukaryotes and prokaryotes are synthesized as preproteins with NH₂-terminal hydrophobic signal sequences which are removed during transfer of the proteins across the membrane^{1,2}. The role of cleavable signal sequences in the insertion of proteins into the cytoplasmic membrane is not clear. Rothman and Lenard have proposed that the assembly of those integral membrane proteins that have a substantial extracytoplasmic domain (ectoproteins) occurs by a similar mechanism to that of secreted proteins, involving a cleavable signal sequence, but with complete secretion being prevented by a hydrophobic COOH-terminal region³. In eukaryotes, several ectoproteins seem to be synthesized in this way⁴⁻⁶ but in prokaryotes, the coat protein of M13 phage is the only reported example of a cytoplasmic membrane protein that is made as a preprotein^{2,7}, although a non-cleavable signal-like sequence has been shown for the *E. coli* lactose permease⁸. We show here that penicillin-binding proteins 5 and 6 of *Escherichia coli* are the first examples of cytoplasmic membrane proteins, encoded by bacteria, that are processed.

Penicillin-binding proteins (PBPs) catalyse the final stages of peptidoglycan synthesis⁹ and in *E. coli* all the seven PBPs have been located exclusively in the cytoplasmic membrane on the basis of both Sarkosyl solubility¹⁰ and their recovery with cytoplasmic membrane fractions on sucrose gradients¹¹. Plasmid pLG310 (ref. 9) carries the PBP6 gene (*dacC*) inserted on a 5.7-kilobase *EcoRI* fragment in the vector pSF2124. Minicells prepared from *E. coli* DS410 (pLG310) synthesized a protein which migrated on SDS-polyacrylamide gels with ¹⁴C-benzylpenicillin-labelled PBP 6 (Fig. 1a, b). As expected, PBP 6 made in minicells was recovered exclusively in the cytoplasmic membrane (data not shown). However, a protein with the molecular weight (MW) of PBP 6 was not detected when pLG310 DNA was used to programme an *in vitro* coupled transcription-translation system, but a protein (PBP 6*) with an apparent MW 3-4,000 larger than PBP6 was synthesized (Fig. 1d). PBP 6* made *in vitro* and PBP 6 made in minicells were shown to be closely related, as several of the ³⁵S-methionine-labelled peptides obtained by partial proteolysis with staphylococcal V8 protease were identical (Fig. 2). Moreover, we were able to show that the addition of inverted cytoplasmic membrane vesicles of *E. coli* during *in vitro* transcription-translation of pLG310 DNA resulted in the appearance of a protein which co-migrated with authentic PBP 6, with the concomitant disappearance of PBP 6* (Fig. 1c). Subsequent recovery of the inverted cytoplasmic membrane vesicles from the incubation mixture showed the *in vitro* processed form to be associated with the vesicles (data not shown), and the peptides obtained after partial proteolysis of this form were identical to those obtained from PBP 6 synthesized in minicells (Fig. 2; the single peptide difference seen in tracks a and b of Fig. 2 disappeared with higher protease concentrations). We conclude from the above evidence that PBP 6* is a preprotein form of PBP 6 and that processing *in vitro* is occurring in an analogous way to that *in vivo*.

The other major protein synthesized *in vitro* from pLG310 DNA was identified as the preprotein form of the periplasmic

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TEM β -lactamase¹² (the pSF2124 vector contains transposon Tn3) by precipitation with antiserum directed against the mature form of this protein. In minicells carrying pLG310, both the mature form of β -lactamase and a small amount of the preprotein form were detected (Fig. 1b). *In vitro* processing of β -lactamase was very poor in most experiments for unknown reasons.

Similar results to those obtained with PBP 6 were obtained when the expression of the PBP 5 gene (*dacA*) was examined *in vitro* and *in vivo*. The *dacA* gene is carried on the specialized transducing phage λ pBS10 (ref. 13). Infection of UV-irradiated *E. coli* 159 (λ imm⁴³⁴) with λ pBS10 resulted in the synthesis of a protein that co-migrated on SDS-polyacrylamide gels with ¹⁴C-benzylpenicillin-labelled PBP 5 and the protein was recovered exclusively in the cytoplasmic membrane of the infected cells

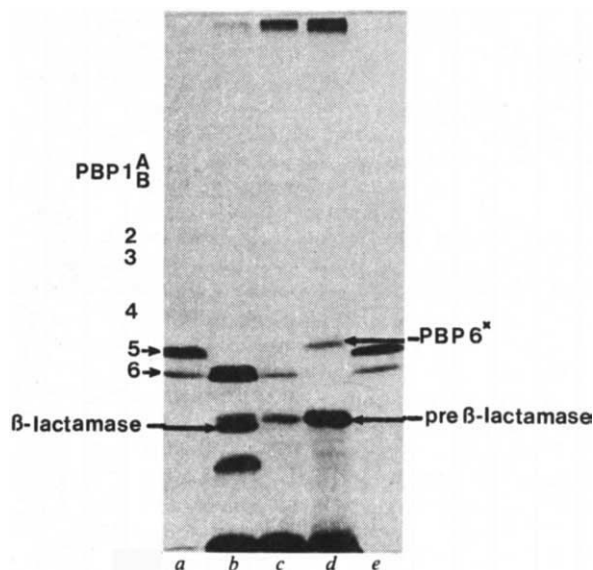


Fig. 1 SDS-polyacrylamide gel electrophoresis of ³⁵S-methionine-labelled proteins synthesized *in vivo* and *in vitro* from pLG310 plasmid carrying the *E. coli* PBP 6 gene. *In vitro* labelled proteins were synthesized in a coupled transcription-translation system based on that of Zubay¹⁹ using *E. coli* MRE 600 to prepare the S30 extract. Inverted cytoplasmic membrane vesicles, which had been treated with EDTA to remove ribosomes, were prepared from *E. coli* ML30 exactly as described by Chang *et al.*⁷. Proteins synthesized *in vivo* from pLG 310 were obtained by incubation of minicells prepared from *E. coli* DS410 (pLG310) with ³⁵S-methionine. *E. coli* PBPs were labelled by incubating cell envelopes of *E. coli* KN126 with ¹⁴C-benzylpenicillin as described elsewhere¹⁰. The labelled proteins were fractionated on a 10% SDS-polyacrylamide slab gel and were detected by fluorography¹⁰. a, e, *E. coli* PBPs labelled with ¹⁴C-benzylpenicillin; b, proteins synthesized in minicells carrying pLG310; c, d, proteins synthesized *in vitro* from pLG310 DNA in the presence (c) and absence (d) of inverted cytoplasmic membrane vesicles. Some of the PBPs in a and e are not visible at this exposure of the fluorograph.

(Fig. 3c and ref. 13). Addition of λ pBS10 DNA to the *in vitro* transcription-translation system resulted in the appearance of a protein (PBP 5*) with an apparent MW 3–4,000 larger than PBP 5 but no protein which co-migrated with authentic PBP 5 (Fig. 3a, b). PBP 5* was concluded to be a preprotein form of PBP 5 by comparing the peptides obtained by partial proteolysis and by showing *in vitro* processing of PBP 5* to PBP 5 (data not shown).

PBPs 5 and 6 are the only examples of cytoplasmic membrane proteins, encoded by bacteria, which are known to be processed. Although we assume that processing of PBPs 5 and 6 occurs by the removal of a hydrophobic NH₂-terminal signal sequence, this has yet to be established.

PBPs 5 and 6 are D-alanine carboxypeptidases which remove the terminal D-alanine residues from the pentapeptide side chains of peptidoglycan⁹. As the removal of D-alanine residues

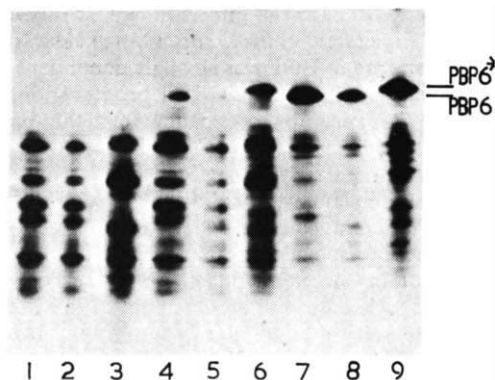


Fig. 2 Peptide mapping of PBP 6 and PBP 6*. Slices of SDS-polyacrylamide gel containing ³⁵S-methionine-labelled PBP 6 processed *in vivo*, PBP 6 processed *in vitro* and PBP 6*, were obtained from the samples used in tracks b, c and d of Fig. 1, respectively. Tracks 1–3, 4–6, 7–9 were partially digested with 0.75 μ g, 0.12 μ g and 0 μ g respectively of *Staphylococcus aureus* V8 protease as described by Cleveland *et al.*²⁰ and the resulting peptides were fractionated on an 18% SDS-polyacrylamide gel and detected by fluorography. 1, 4, 7, PBP 6 processed *in vitro*; 2, 5, 8, PBP 6 processed *in vivo* minicells; 3, 6, 9, PBP 6*. Slight proteolysis in tracks 7–9 is due to overspill of protease from other tracks.

continues after the release of nascent peptidoglycan from the cytoplasmic membrane, and after its attachment to pre-existing peptidoglycan¹⁴, it is likely that PBPs 5 and 6 are ectoproteins with the bulk of their polypeptides extending into the periplasmic space. There are little data on the membrane orientation of PBPs 5 and 6 of *E. coli* but there is good evidence that the analogous PBP 5 of *Bacillus subtilis* (and *Bacillus stearothermophilus*) is an ectoprotein embedded in the membrane by a hydrophobic COOH-terminal peptide, with the bulk of the polypeptide soluble outside the membrane¹⁵. The synthesis of PBPs 5 and 6 as preproteins is consistent with the view that they are also ectoproteins and that those proteins, where the bulk of

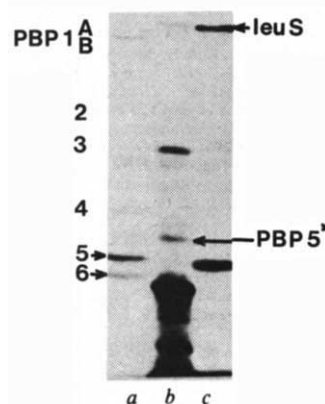


Fig. 3 Synthesis of *E. coli* PBP 5 *in vitro* and *in vivo* from λ pBS10. ³⁵S-methionine-labelled proteins were fractionated on a 10% SDS-polyacrylamide gel and detected by fluorography. a, PBPs of *E. coli* KN126 labelled with ¹⁴C-benzylpenicillin; b, ³⁵S-methionine-labelled proteins synthesized *in vitro* from λ pBS10 DNA, as described in Fig. 1 legend; c, ³⁵S-methionine-labelled proteins synthesized following infection of UV-irradiated *E. coli* 159 (λ imm⁴³⁴) with λ pBS10 as described previously¹³. leuS = leucyl-tRNA synthetase encoded by the *leuS* gene carried on λ pBS10.

the polypeptide must pass across the membrane, are synthesized with cleavable signal sequences in both prokaryotes and eukaryotes. Whereas cytoplasmic membrane proteins may in some cases be made with signal sequences (for example the lactose permease⁸), the need to remove the sequence may only arise for ectoproteins in which the NH₂-terminal region is required to extend away from the membrane. This may be particularly important for PBPs 5 and 6 because the substrate-binding site of PBPs that have been examined is extremely close to the NH₂-terminus of the protein^{16,17}.

Recently, considerable similarities have been found between PBPs and β -lactamases and it has been suggested that these proteins evolved from a common ancestral penicillin-sensitive enzyme involved in peptidoglycan synthesis^{17,18}. In the light of the present data PBPs 5 and 6 can be considered as pseudo-periplasmic proteins which are secreted through the membrane by the same mechanism as the periplasmic β -lactamases, until

synthesis of a hydrophobic COOH-terminal peptide prevents their complete release as soluble proteins into the periplasm. It can be envisaged that a PBP involved in peptidoglycan synthesis, which already catalysed a weak β -lactamase reaction, could have evolved into a periplasmic β -lactamase by amino acid substitutions that increase the rate of deacylation of bound penicillin, and by deletions that result in the loss of the COOH-terminal hydrophobic membrane-anchoring peptide. It should be possible to mimic these proposed evolutionary steps by *in vitro* manipulation of the cloned PBP 5 or PBP 6 genes.

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Expression of Tn9-derived chloramphenicol resistance in *Bacillus subtilis*

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The ability of cells to discriminate between homologous and foreign genes manifests itself differently from one species to the next. *Escherichia coli* is well known for its ability to express genes from various donor species, including *Bacillus subtilis*^{1,2}. In contrast, *B. subtilis* strongly discriminates against the expression of *E. coli* genes (there have been no corroborated reports of the expression of a wholly intact *E. coli* gene in *B. subtilis*) but expresses genes from other Gram-positive bacteria such as *Staphylococcus aureus*³. This apparent asymmetry in the expression of heterologous genes between *E. coli* and *B. subtilis* may reflect fundamental differences in the mechanisms of gene expression in these two model systems. Here, we have overcome the inability of *B. subtilis* to express *E. coli* chloramphenicol resistance (Cm^r) by supplanting the native regulatory element(s) of this Tn9-derived gene with *B. subtilis* DNA fragments, which represents the first step towards dissecting the nature of this asymmetric barrier to gene expression.

Most bacterial resistance to chloramphenicol is due to a chloramphenicol acetyl transferase (CAT) encoded on R-factors in Gram-negative⁴ and Gram-positive bacteria^{5,6}. In Gram-negative bacteria, the Cm^r gene on the transposable genetic element Tn9 codes for a 219-amino acid protomer (EC 2.3.28). In solution, Tn9-derived CAT exists as a homotetramer⁷. The Cm^r gene from Tn9 has been transferred to a plasmid cloning vector, designated pBR328 (ref. 8). Using pBR328 as a source of the Cm^r gene, Close and Rodriguez (in preparation) have isolated the structural portion of the Cm^r gene on a 774-base pair (bp) *TaqI* restriction fragment. The CAT-containing *TaqI* fragment deletes the transcriptional promoter but retains the putative ribosomal binding site within a short leader sequence (Fig. 1). The *TaqI* ends of the 774-bp fragment were converted to either *HindIII* or *SalI* ends, and cloned into pBR327 to give pCM7 and pCM1 respectively.

Table 1 CAT activity in crude extracts of *B. subtilis* BD630

Clone	Insert (bp)	Cm ^r ($\mu\text{g ml}^{-1}$)	<i>B. subtilis</i> CAT activity†
BD630	—	—	0
pEB1	—	—	0
pGR71	—	—	0
pGR71-4	1,630*	15	241
pGR71-7	1,390	15	201
pGR71-8	920	25	176
pGR71-12	1,860	35	588
pGR71-15	1,200	15	169
pGR71-36	480	5	62
pGR71-38	1,200	35	508
pGR71-43	1,400*	45	1900
pGR71-49	900	15	122
pGR71-64	1,600*	<5	21
pGR71-65	790	20	244
pGR71-82	1,350*	10	93
pGR71-91	1,050	30	719
pGR71-104	996	30	212
pGR71-111	360	5	14
pGR71-121	720	25	316
pGR71-125	1,250	5	93
pGR71-126	1,100	20	93
pGR71-131	650	20	296
pGR71-140	1,160*	10	38

Chloramphenicol acetyl transferase (CAT) activity, expressed as nmol per min per mg protein, was determined spectrophotometrically as described by Shaw⁹. Plasmid-harboring strains were grown overnight in brain-heart infusion broth containing 10 $\mu\text{g ml}^{-1}$ kanamycin or 5 $\mu\text{g ml}^{-1}$ chloramphenicol. Cells were washed with 1 M NaCl, 50 mM Tris-HCl, pH 7.9. The cell pellet was suspended in 1.5 ml 50 mM Tris-HCl, pH 7.9, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 50 μM dithiothreitol (DDT), 0.5 mg ml⁻¹ lysozyme. This suspension was incubated at 37 °C until lysis occurred. The crude extract was briefly sonicated to shear viscous chromosomal DNA and centrifuged in an Eppendorf microfuge for 15 min. The supernatant was first diluted with 50 mM Tris-HCl, pH 7.9, 0.1 mM PMSF, 50 μM DTT or directly assayed. Total protein was determined by the Coomassie blue method¹⁴.

* Insert has an internal *HindIII* site.

The plasmid pGR71, an *E. coli*/*B. subtilis* co-integrate, was constructed specifically for use in these studies (Fig. 1). pGR71 confers kanamycin resistance (Km^r) on both hosts and low-level Cm^r (5 $\mu\text{g ml}^{-1}$ chloramphenicol in solid medium) in *E. coli*. *B. subtilis*, transformed with pGR71, is chloramphenicol sensitive and contains no CAT activity as determined by colorimetric assay⁹. The low-level expression of Cm^r in *E. coli* is probably due to read-through transcription from promoters upstream of the Cm^r gene. A second co-integrate plasmid, pEB1 (Fig. 2), contains the intact Cm^r gene and thus confers high-level Cm^r on *E. coli* but not on *B. subtilis* (Table 1).

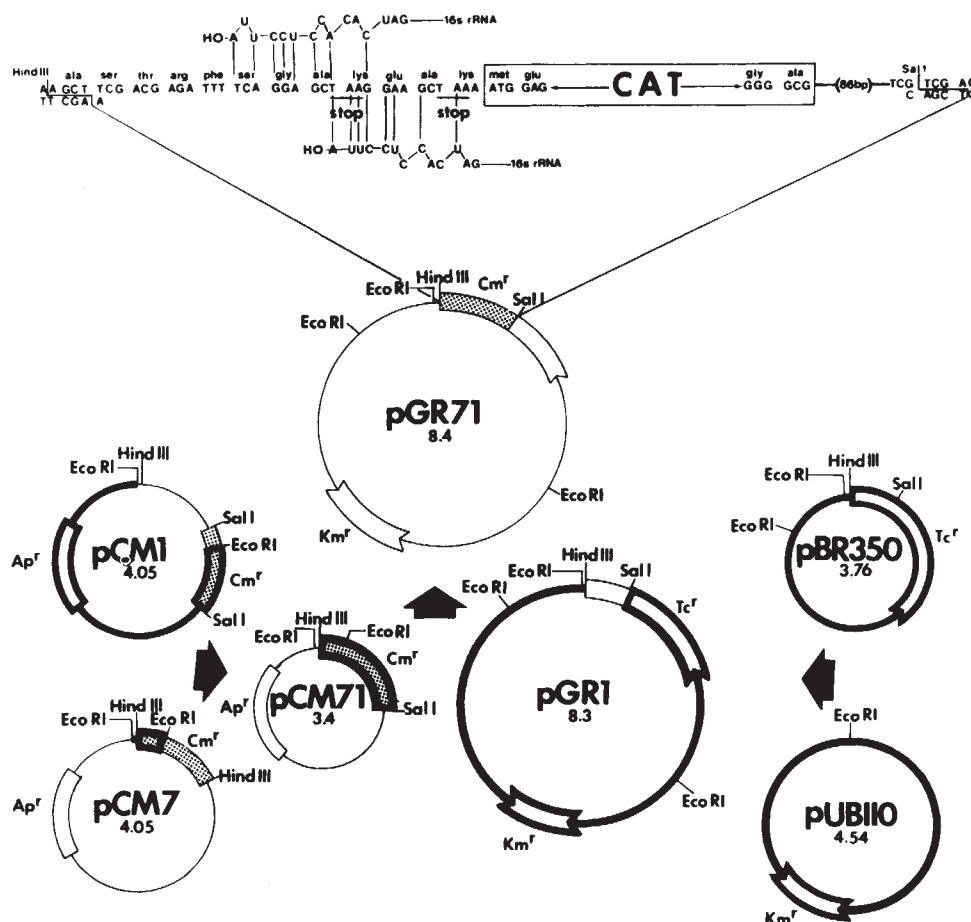


Fig. 1 A schematic representation of the construction of pGR71 and the substructure of the CAT gene within it. The lower portion of the figure shows the strategy used to construct pGR1, pCM71 and pGR71. The shaded area of each parent plasmid represents the restriction fragment used to construct the new plasmid derivative. The rationale underlying the construction of pGR71 was to introduce the CAT gene into a co-integrate plasmid—pGR71—so that the gene is directly preceded by a unique *Hind*III site, which can be used to clone exogenous *Hind*III-generated DNA fragments. The plasmids pCM1 and pCM7 were constructed from pBR328 and pBR327 (Close and Rodriguez, in preparation). pUB110 has been isolated and described elsewhere³. The 786-bp *Hind*III–*Sal*I CAT-containing fragment was derived from a *Taq*I fragment starting from the TCGA sequence at the left to the TCGA sequence at the right¹⁵. Two translational stop codons, both outside the reading frame for the CAT protein, are indicated. Two sequences showing extensive homology with the 3' end of the *E. coli* 16S rRNA are also represented¹⁵. Hypothetically, translational initiation can begin within a cloned *Hind*III-cleaved DNA insert, or from the AUG codon adjacent to the putative ribosomal binding sites within the 36 nucleotides between the AUG codon and the *Hind*III cloning site on the DNA. Translational initiation within a cloned *Hind*III fragment should continue through the leader sequence and into the structural gene without termination.

We activated the Tn9-derived Cm^r gene by insertion of *B. subtilis* chromosomal DNA fragments in the following way. A 1:1 mass ratio of *Hind*III-cleaved *B. subtilis* BD630 chromosomal DNA and pGR1 were ligated using a final total DNA concentration of 100 ng μ l⁻¹. When transforming *B. subtilis* directly with the ligation mixture, transformants were first selected for the presence of plasmid on kanamycin plates and then replica-plated on to agar plates containing 5 μ g ml⁻¹ chloramphenicol. Table 1 shows some of the properties of recombinant plasmids isolated from Cm^r *B. subtilis* clones using the protocol described above. All these plasmids were found to be stable to deletion in *B. subtilis* BD630 and *E. coli* HB101. As pEB1 was derived from pBR328, which may undergo spontaneous deletion in *E. coli*¹⁰, only freshly transformed host cells were used in our experiments.

Both *in vivo* (efficiency of plating) and *in vitro* (CAT activity assay) determinations of Cm^r provide reproducible estimates of gene expression. The colorimetric assay for CAT is sensitive over at least four orders of magnitude (1.0–12,000 units per min per mg protein, data not shown). Therefore, while plating efficiencies were used to identify Cm^r transformants, CAT activity was taken as a more direct measure of Cm^r gene expression. All pGR71 derivatives which confer chloramphenicol resistance on *B. subtilis* were also capable of conferring at least low-level Cm^r on *E. coli*. When *E. coli* was directly transformed with the ligation mixture and selected for Cm^r and Km^r, it was found that *B. subtilis* DNA could directly activate the Cm^r gene of pGR71 in *E. coli*. Plasmids isolated from Cm^r *E. coli* after transformation always contain pGR71 with *Hind*III insert(s). Interestingly, not all these plasmids were capable of transforming *B. subtilis* to Cm^r even though the plasmids were stable to deletion and curing.

Although the plasmid copy numbers for the different Cm^r derivatives of pGR71 have not been determined directly, we believe them to be similar on the basis of the levels of Km^r as determined by growth rate in selective medium (data not

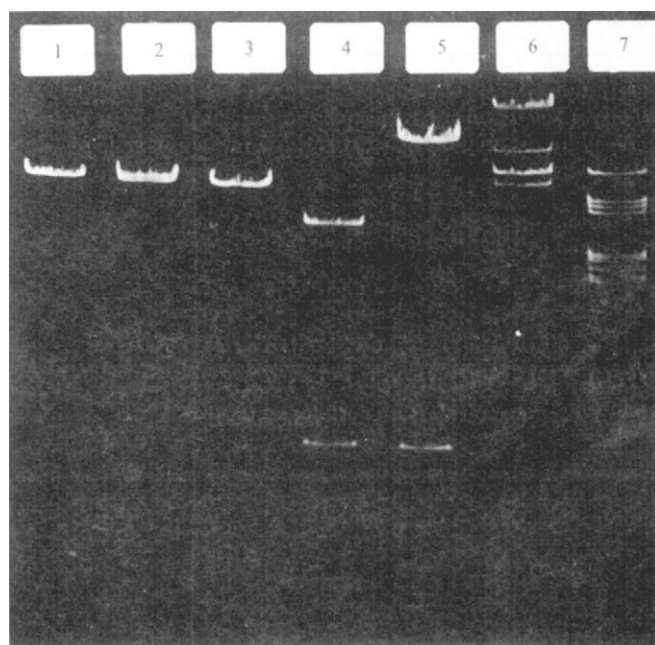


Fig. 2 Agarose (1%) gel electrophoresis of plasmids was used. pEB1 was obtained by cleaving pBR328 and pUB110 with *Bam*HI and ligating with T4 DNA ligase. Transformants of *E. coli* HB101 and *B. subtilis* BD630 were subsequently selected for a Km^r, Cm^r co-integrate plasmid. 1, *Bam*HI-cleaved pBR328; 2, *Bam*HI-cleaved pEB1; 3, *Bam*HI-cleaved pUB110. The ~780-bp DNA fragment containing the CAT structural gene is represented by a *Hind*III–*Sal*I double digest of pCM7 (4) and *Hind*III–*Sal*I double digest of pGR71 (5). Lanes 6 and 7 show *Eco*RI-cleaved λ DNA and *Hpa*-I cleaved T7 DNA standards. T7 and λ DNAs were gifts from M. Chamberlin and T. Close respectively. pUB110 was isolated from wild-type *B. subtilis* 168 by a procedure described elsewhere¹⁶. All other plasmid DNAs were purified either from *E. coli* HB101 or from RR1 as previously described¹⁷.

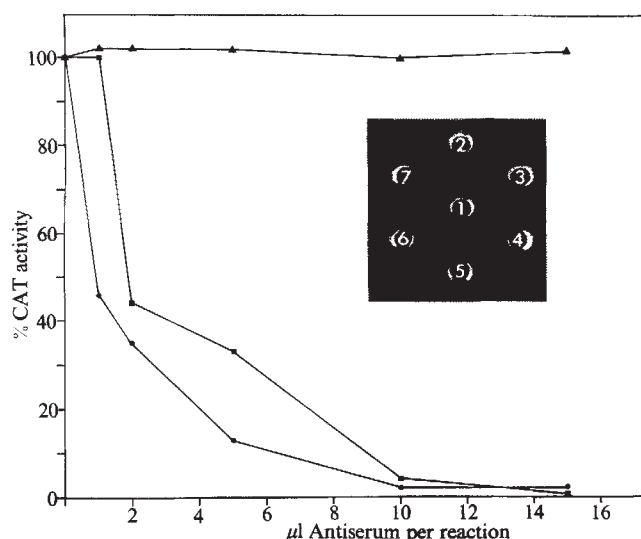


Fig. 3 Specific immunological inhibition of CAT activity. Crude antiserum to purified *E. coli* pBR328-encoded CAT was raised by immunizing a New Zealand White rabbit. Milligramme quantities of *E. coli* CAT were purified by the second ammonium sulphate precipitation described elsewhere⁹ except that 0.1 mM PMSF was present in the breaking buffer. The ammonium sulphate pellet was suspended and dialysed overnight against 50 mM Tris-HCl pH 7.9, 50 μ M DTT, 0.1 mM PMSF and 0.2 mM chloramphenicol before loading on to a 20-ml coenzyme A (CoA) linked Affigel 10 column. The affinity column coupling reaction was performed overnight at 4 °C in a closed plastic tube using 20 ml Affigel 10 (Bio-Rad) and 52 mg CoA (Sigma) in 10 mM 2-(*N*-morpholino)ethanesulphonic acid (MES buffer), pH 6.0. 1.85 mg CoA was coupled per ml of Affigel 10, as determined by the coupling efficiency (73%) of a ³H-labelled CoA (NEN) tracer, added with the unlabelled coenzyme A. The loaded column was washed with two column volumes of 50 mM Tris-HCl pH 7.9, 50 mM NaCl and 50 μ M DTT, then developed with 100 ml 0.05–0.5 M NaCl linear gradient in the same buffer. The CAT activity peak eluted at 0.3 M NaCl. Fractions containing pure CAT, as judged by SDS-electrophoresis, were pooled, the volume reduced and stored at –20 °C. Inactivation by anti-CAT antiserum of crude extracts (prepared as described in Table 1 legend) was monitored by the spectrophotometric method of Shaw⁷ as follows: 5–10 μ l of purified enzyme or crude extract and 0–15 μ l antiserum were added on ice to a 1-ml complete CAT assay reaction mix (0.4 mg ml^{–1} 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), 100 mM acetyl CoA, 0.1 mM chloramphenicol, 50 mM Tris-HCl, pH 7.9). The reaction mix was left on ice for 10 min and then transferred to a 37 °C water bath. After a 5-min incubation at 37 °C, CAT activity was determined by absorbance at 412 nm. ●, Purified CAT from *E. coli* pBR328; ■, crude extract of *B. subtilis* BD630 harbouring pGR71-15; ▲, crude extract of *B. subtilis* BD630 harbouring pC194. Inset shows a double-diffusion Ouchterlony test of 15 μ l anti-*E. coli* CAT antiserum (well 1) with 6.8 mg of purified *E. coli* CAT (wells 4 and 7); 15 μ l crude extract of BD630 harbouring pGR71-15 (wells 2 and 5); 15 μ l crude extract BD630 (well 3) and 15 μ l crude extract of BD630 harbouring pC194 (well 6). The 1% agarose Ouchterlony plate was incubated overnight at room temperature before the photograph was taken.

shown). A direct correlation between plasmid copy number and level of antibiotic resistance has been established previously¹¹. In addition, the fact that similar amounts of plasmid DNA have been isolated from different clones suggests that copy number is not greatly affected by the presence of *Hind*III inserts. Therefore, we conclude that the different levels of CAT activity exhibited by the pGR71 derivatives are probably due to differential gene expression and not to differences in plasmid copy number.

To establish that the *B. subtilis* Cm^r phenotype was due to Tn9-derived CAT activity and not, for example, to a contaminating Gram positive-derived Cm^r gene or a chromosomal mutation, crude cellular extracts were prepared from Cm^r, Km^r *B. subtilis* transformants and when assayed (Table 1), all were found to contain some level of CAT activity. The lack of immunological cross-reactivity between Gram-positive and Gram-negative Cm^r gene products^{5,6} enabled us to demonstrate the Gram-negative origin of the CAT activity in Cm^r, Km^r *B. subtilis* clones. Double-diffusion Ouchterlony (Fig. 3) experiments showed lines of identity between the *E. coli* pBR328 and *B. subtilis* pGR71-15 CAT enzymes. Antiserum did not visibly cross-react with crude extracts of BD630 or BD630 harbouring pC194. pC194 carries a Cm^r originally

isolated from *S. aureus*¹² and is immunologically representative of CAT found in Gram-positive bacteria⁶. Antiserum to *E. coli* CAT was shown specifically to inhibit both *E. coli* pBR328-encoded CAT and the CAT produced by a specific Cm^r, Km^r *B. subtilis* clone, pGR71-15, but not that produced by pC194-transformed *B. subtilis* (see Fig. 3). The CAT activity in crude extracts of other Cm^r, Km^r *B. subtilis* was also specifically inhibited by anti-CAT antiserum (data not shown).

The level of Cm^r on agar plates and CAT activity in crude extracts varied among the different *B. subtilis* transformants, depending on the *Hind*III insert(s). However, there was no correlation between the level of Cm^r and the DNA insert size (Table 1). The ability of different fragments to determine the level of Cm^r gene expression supports the notion that expression is directed by *B. subtilis* promoters and possibly by translational initiation signals within the cloned fragments. Because the putative Gram-negative ribosomal binding site for the Cm^r gene is still present in pGR71 (Fig. 1), we cannot be certain whether translation is initiating from within the DNA insert or from the native site. However, the inability of *in vitro* *B. subtilis* translation systems to translate *E. coli* mRNA¹³ supports the notion that *in vivo*, a *B. subtilis* ribosomal binding site must be supplied with a promoter to allow gene expression. We are now studying the nature of gene expression of the activated Cm^r gene inserted in *B. subtilis* and *E. coli*.

From the above evidence, we conclude that although the native *E. coli*-derived Cm^r gene is not expressed in *B. subtilis*, the structural portion of this gene can be expressed when controlled by *B. subtilis* regulatory elements. Finally, as *B. subtilis* DNA inserts can activate the expression of the Cm^r gene, it should be possible to use pGR71 or a similar plasmid as a gene-expression vector in *B. subtilis* and other related Gram-positive hosts.

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Regulation of protein synthesis during heat shock

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When the cells or tissues of most eukaryotes are exposed to elevated temperatures, they respond with the vigorous induction of a small number of 'heat shock' proteins (hsps). I report here investigations on the responses of two very different organisms, the fruit fly *Drosophila melanogaster* and the yeast *Saccharomyces cerevisiae*. Although both organisms achieve a very rapid shift in protein synthesis, they do so in very different ways. In *Drosophila*, heat shock induces a mechanism of translational control which both promotes the translation of hs mRNAs and specifically represses the translation of pre-existing mRNAs. Yeast cells, in contrast, do not possess a special mechanism to sequester pre-existing messages from translation. Instead, most of these messages simply disappear rapidly from the cell, while those that are retained continue to be translated.

Heat treatment of *Drosophila* cells induces drastic changes in transcription, with immediate inhibition of normal mRNA synthesis and vigorous induction of hs mRNAs¹⁻⁵. However, these changes alone cannot account for the radical changes which take place in protein synthesis. Heat shock must also activate some mechanism to clear pre-existing mRNAs from polysomes. This is apparent from the simple fact that normal polysomes disappear before hs mRNAs accumulate, and disappear even when hs mRNA synthesis is blocked⁶. One way to clear the cell of polysomes would be to degrade their messengers. An argument against this simple explanation is the fact that translational activity for normal proteins can be recovered from heat-shocked cells (assayed *in vitro*)⁷⁻⁹. Unfortunately, such experiments are difficult to quantify satisfactorily due to the presence of many RNAs which normally are not translated efficiently in *Drosophila* cells¹⁰. There is another very simple way to confirm the retention of pre-existing messages in heat-shocked cells. As shown in Fig. 1a, cells recover the full spectrum of normal protein synthesis when returned to normal temperatures, even when new transcription is blocked with actinomycin^{8,11}. Control experiments (not shown) demonstrated that the drug effectively blocked mRNA synthesis and did not artificially increase isotope incorporation by shrinking the pool of free leucine. Furthermore, in independent experiments monitoring polysomes after heat shock, normal profiles reappeared with equal speed in the presence or absence of actinomycin. The recovery of protein synthesis in Fig. 1a is therefore almost certainly due to resumed translation of pre-existing mRNAs.

The ability of normal messages to return to translation after heat shock is remarkably reproducible. For Fig. 1b, cells were heat shocked, treated with actinomycin and allowed to recover at 25 °C, followed by three further cycles of treatment. Normal messages were repeatedly sequestered during heat shock and re-used during recovery. Note that synthesis of hsps dampens out during each recovery. This finding provides an internal control that actinomycin is effectively blocking new transcrip-

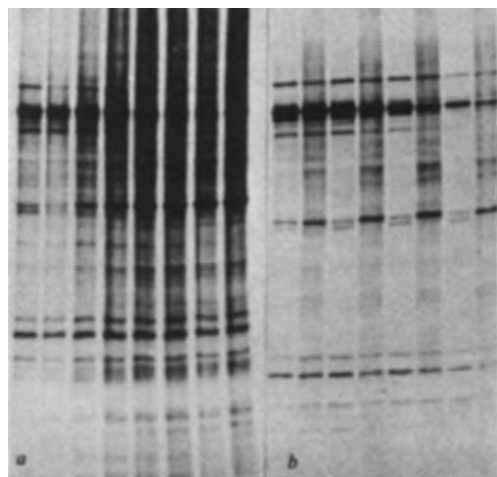


Fig. 1 Retention of normal cellular messages in heat-shocked cells, demonstrated by the recovery of protein synthesis in the presence of actinomycin D. *a*, Time course of recovery after a 15-min heat shock. Identical aliquots of cells were incubated in a 37 °C water bath for 15 min. Actinomycin D was then added ($1 \mu\text{g ml}^{-1}$) and cells were returned to 25 °C. Individual aliquots were pulse-labelled with ^3H -leucine for 15 min in consecutive 15-min intervals. The complete spectrum of normal protein synthesis is restored despite the inhibition of new transcription with actinomycin D. *b*, Repeated heat shock-recovery cycles. Identical aliquots of cells were incubated for 25 min at 37 °C. Actinomycin D was added ($1 \mu\text{g ml}^{-1}$) and the cells allowed to recover for 90 min at 25 °C. The cells were then subjected to three additional cycles of treatment at 37 °C for 25 min followed by 90 min of recovery at 25 °C. One aliquot was labelled with ^3H -leucine during the last 10 min of each temperature shift. Normal cellular messages are repeatedly sequestered from translation during heat shock but return to protein synthesis during recovery. Proteins were analysed on 11% polyacrylamide-SDS slab gels¹⁹ which were impregnated with fluor²⁰ and autoradiographed with preflashed XR-5 film. Each slot contains protein from 10^5 cells.

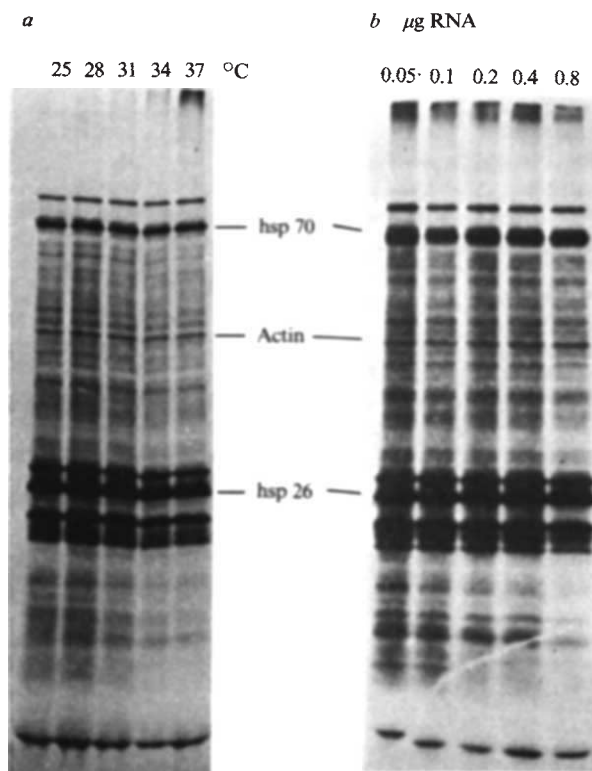


Fig. 2 Effects of changing temperature or RNA concentration on the translation of *Drosophila* messages *in vitro*. Poly(A)-containing polysomal RNA from heat-shocked and control cells⁵ was mixed together in equal quantities and translated in a nuclease-cleared reticulocyte lysate²¹. To compensate for differences in overall incorporation, equal c.p.m.s from each same sample were layered on the gel. *a*, Translation of 0.2 µg RNA at various temperatures. Temperature has little effect on the relative translational efficiencies of heat-shock and control messages. *b*, Translation over a 16-fold range of RNA concentration at 33 °C. Incorporation was linear with respect to RNA concentration in the range of 0.01–0.15 µg RNA per 15 µl reaction. Heat-induced messages do not have a major advantage for initiation when the translational capacity of the lysate becomes limiting. All reactions were performed in 15 µl with 150 mM KAc and 20 mM MgAc.

tion (in its absence each heat shock induces new hs mRNA and protein synthesis). It also indicates that the mechanism which protects untranslated messages during heat shock is highly specific; normal messages are protected during heat shock but hs mRNAs are not protected during recovery.

The fact that *Drosophila* cells preferentially translate hs mRNAs at high temperatures does not necessarily mean the process is a regulated one. Temperature-induced changes in the secondary structure of mRNAs can critically affect their relative translational efficiencies¹². To determine whether the change in translation during heat shock is the simple consequence of such effects, mRNAs from 25 °C cells and from matched heat-shock cultures were mixed and translated *in vitro* at 25, 28, 31, 34 and 37 °C (Fig. 2a). Clearly, temperature has only a very minor effect on the relative translational efficiencies of these RNAs. Similar results have been obtained with total polysomal RNA and with poly(A)-containing RNA, with RNA concentrations adjusted above or below the level of saturation. Another laboratory has found that, when supplied with a mixture of control and hs mRNAs and incubated at intermediate temperatures, control cell-free translation lysates from *Drosophila* cells¹³ produced both types of protein, while hs lysates produced primarily hsps. Thus the different patterns of protein synthesis in heat-shock and control cells reflect a very real difference in their translational activities.

Before we can conclude that *Drosophila* has a truly selective mechanism of translational regulation, one more possibility must be eliminated. As Lodish has pointed out¹², any nonspecific reduction in initiation will result in translation of only those messages which have a particularly high affinity for ribosomes. Profound changes in the patterns of synthesis can

thus be achieved without a real change in the specificity of translation. If heat treatment reduces the rate of initiation, the changed pattern of synthesis might simply be due to hs mRNAs having a competitive advantage for a limiting resource.

This question has been investigated both *in vitro* and *in vivo*. In the experiment presented in Fig. 2b, a mixture of control and hs mRNAs were translated at various concentrations in a reticulocyte lysate. At low concentrations of mRNA, both populations should be translated efficiently. As the concentration reaches saturation (at 0.2 μg of RNA), messages with an advantage for initiation should dominate the reaction. Although certain proteins do drop out as RNA concentration increases, hs mRNAs do not have a major advantage for initiation with respect to the sum total of normal messages. A different approach is to measure the actual rates of initiation *in vivo*. As we have reported elsewhere¹⁴, only hs messages were readily amenable to this type of analysis. Nevertheless, their rates of initiation were comparable with the fastest known rates in other eukaryotic systems. Thus, if some factor does become limiting for translation of normal cellular messages, it must not be limiting in the same way for heat-shock mRNAs.

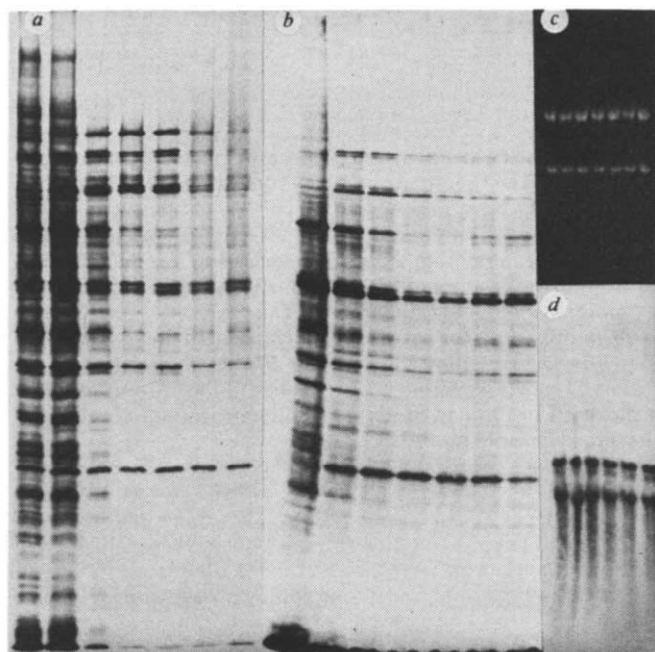


Fig. 3 Changing patterns of translation during heat shock in yeast. *a*, Time course of protein synthesis *in vivo*. A culture of yeast cells (A364a) growing in minimal dextrose medium at 25 °C with vigorous aeration was transferred to a prewarmed Erlenmeyer flask shaking in a 40 °C water bath. Fifteen minutes before transfer, and in 15-min intervals thereafter, 100- μl aliquots were removed from the culture to prewarmed test tubes containing ^3H -leucine. After a 15-min labelling, cells were diluted with 3 ml of ice-cold sorbitol, collected by centrifugation, treated briefly on ice with 50% v/v glucosylase sorbitol and washed in sorbitol again. Proteins were solubilized in SDS sample buffer and electrophoresed on a 12% SDS-polyacrylamide slab gel. *b*, *In vitro* translation of RNAs isolated from the same cell culture. At the midpoint of each protein-labelling period (that is, 7.5, 22.5, 37.5, 52.5, 67.5 and 82.5 min of heat treatment), 50 ml of culture were removed from the flask and collected by centrifugation. The cells were resuspended in 2.5 ml of ice-cold extraction buffer (0.1 M Tris pH 7.5, 0.1 M LiCl, 0.01 M dithiothreitol) and added to a vigorously vortexing emulsion of 0.5 ml 10% SDS, 2.5 ml phenol, 2.5 ml chloroform and 7 g of glass beads. Vortexing was continued without interruption for 5 min. The phases were separated by centrifugation and the aqueous phase was re-extracted twice with phenol/chloroform. RNA was precipitated four times with ethanol. Translation of total cellular RNA in a reticulocyte nuclease-cleared lysate²¹ was performed at a concentration of 120 $\mu\text{g ml}^{-1}$ in 150 mM KAc and 1 mM MgAc at 33 °C. *c*, *d*, Electrophoretic analysis of the RNA preparations translated in *b*. An equal quantity of each sample (2 μg per slot) was electrophoresed on methyl mercury agarose gels¹⁷. *c*, Ethidium bromide fluorescence shows equal recovery of RNA in each preparation and a ratio of 2:1 for the large and small yeast rRNAs. *d*, After impregnation of the gel with diphenylxazole in dimethyl sulfoxide: Dioxane, the autofluorogram shows uniform recovery of ^3H -labelled *Drosophila* RNA (the ribosomal precursor and small subunit RNA) added to the yeast cells before RNA extraction to monitor degradation.

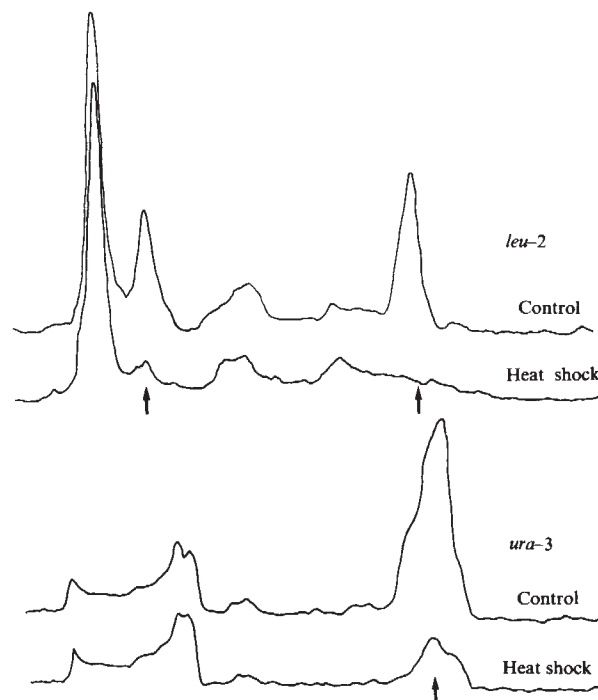


Fig. 4 Disappearance of yeast RNAs during heat shock. Plasmids containing sequences of the *leu-2* (ref. 22) and *ura-3* (ref. 23) genes of *S. cerevisiae* were nick-translated and hybridized to yeast RNA which had been electrophoretically separated on methyl mercury agarose gels and transferred to nitrocellulose filters²⁴. Each well of the gel contained 2 μg of total cellular RNA from the same preparations analysed by *in vitro* translation in Fig. 3b, lanes 1 and 4. Autoradiograms were scanned on a Joyce Loeb microdensitometer; the direction of electrophoresis was from left to right. Three RNA species present in control cells (indicated by arrows) have virtually disappeared from the heat-shock cells. Hybridization on the far left is due to DNA present in the samples.

Yeast cells also respond to temperature elevation with a marked change in protein synthesis¹⁵⁻¹⁷. As with *Drosophila*, a small number of proteins is strongly induced (Fig. 3a). No simple statement, however, can describe the change in pre-existing proteins. A dramatic decline in the synthesis of certain proteins is evident within a few minutes. Others decrease more gradually, while some continue to be synthesized at their initial levels. This is in marked contrast to the *Drosophila* response, in which the pre-existing proteins decline as a cohort, disappearing very rapidly and with the same kinetics.

To determine whether the disappearance of yeast proteins from the synthetic profile is due to translational discrimination, yeast RNAs were isolated and assayed by *in vitro* translation. RNA was extracted from aliquots taken at the midpoint of each protein-labelling interval. The patterns of *in vitro* and *in vivo* synthesis correspond very closely (Fig. 3b), suggesting that reduced synthesis of particular proteins during heat shock correlates with the degradation of their mRNAs. However, it is necessary first to eliminate three other possible explanations of the data.

(1) It is quite reasonable to suppose that messages which are translated inefficiently *in vivo* are more susceptible to degradation during extraction. Their apparent disappearance would then be merely an artefact of isolation. Therefore, an extraction method was devised which allowed rapid and quantitative recovery of undegraded RNA. Using this method, yeast rRNAs gave quantitatively identical fluorescence patterns after gel analysis, with no evidence of breakdown, that is, the ratio of large to small subunit RNA was 2.0 (Fig. 3c). To control for degradation of particularly vulnerable species, a small quantity of purified ^3H -labelled RNA of very high specific activity was added to each cell pellet before extraction. It was recovered completely intact and undegraded in each sample (Fig. 3d).

(2) It is possible that some regulatory factor co-purified with the RNAs and influenced the patterns of translation *in vitro*.

Heat-shock and control mRNAs were therefore mixed and translated together at various temperatures. The results (data not shown) were purely additive, that is, the hs RNAs contained no factor which influenced translation of normal messages.

(3) It is possible that pre-existing messages are physically altered during heat shock in some way which blocks their translation both *in vivo* and *in vitro*. The concentration of particular mRNAs was therefore quantified by hybridization to cloned genes. The RNA samples of lanes 1 (25 °C control) and 4 (52.5 min at 40 °C) in Fig. 3 were analysed by Northern hybridization with *leu-2* and *ura-3* genes (Fig. 4). The major RNA species hybridizing to *ura-3* is ~1 kilobase (kb) long and is reduced fivefold after nearly 1 h of treatment. The *leu-2* plasmid also contained a portion of the *ty-1* sequence. The species on the far right is ~1.2 kb, exactly the size expected to code for the *leu-2* gene product, isopropylamide dehydrogenase. It is reduced to less than 5% of its normal level after heat shock. The large RNA on the left is *ty-1* which virtually disappears during heat shock. (It is intriguing that *ty-1*, which has yet to be associated with a protein-coding function, is also regulated by heat-shock repression.)

The biological assays (by *in vitro* translation) and the physical assays (by hybridization with cloned probes) agree. Most pre-existing mRNAs are degraded during heat shock, although at varying rates. Yeast cells, unlike *Drosophila* cells, do not induce a special mechanism for sequestering pre-existing messages from translation. Many questions remain to be answered. We do not know what changes take place in transcription. Are certain messages retained during heat shock because they continue to be transcribed, or do they simply have much slower turnover rates? Are these the same as their turnover rates at 25 °C or do they change during heat shock? Note also that it is not possible rigorously to exclude translational control from a role in the response. It is conceivable that selected reduction in the translation of certain messages is the signal for their degradation. At any rate, it is clear that the changing patterns of protein synthesis observed in yeast are directly correlated with the changing populations of message in the cell.

The functional significance of the heat-shock response is unknown. Nevertheless, its speed, magnitude and ubiquitous distribution among eukaryotes suggest that the ability to shift rapidly to heat-shock protein synthesis is of critical importance to eukaryotic cells. Because the normal message complement of *Drosophila* cells is long lived¹⁸, even vigorous induction of new message would require several hours to produce a major impact on the protein profile of the cell. This represents a substantial fraction of developmental time in the tightly programmed life cycle of this organism. Experiments presented here, together with reports from other laboratories, offer overwhelming evidence that these cells use a rigorously discriminating mechanism of translational control to maximize their response. The translational machinery of the cell is rapidly cleared for production of hsp's yet the transcriptional investment in pre-existing messages is preserved. In yeast cells, where message half lives are typically measured in minutes²⁵, no such mechanism seems to exist. Instead, pre-existing messages are simply allowed to decay at their own rates, leaving the cell free to shift into heat-shock protein synthesis with nearly the same speed as occurs in *Drosophila*.

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Primary structure of C-terminal functional sites in ovine rhodopsin

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Rhodopsin is the primary photoreceptor protein in the vertebrate retina. The functional complex consists of the polypeptide, opsin, to which is bound a molecule of 11-*cis*-retinal. An absorbed photon of light induces electronic changes in this chromophore, resulting in its isomerization to the all-*trans* form¹ and the triggering of a series of spectrally-defined conformational changes in the protein. This 'activation' of rhodopsin promotes biochemical events which result in the transmission of an integrated response to the brain (for reviews, see refs 2-4). Here we report the amino acid sequence of the C-terminal third of the ovine protein, in which we identify the retinal-binding and phosphorylation sites.

Ovine opsin consists of a single glycosylated polypeptide of apparent molecular weight (MW) 38,000 and constitutes about 90% of the protein contained in the rod photoreceptor membranes. The bulk of the protein is thoroughly embedded in the lipid bilayer, which accounts for the limited susceptibility of the native polypeptide to degradation by proteases⁵⁻⁹. We have made use of this property to cleave ovine rhodopsin efficiently *in situ* with the protease V8 from *Staphylococcus aureus*, an enzyme specific for Glu or Asp-X peptide bonds¹⁰.

Treatment of intact photoreceptor membranes with this protease cleaved the protein into two membrane-bound fragments, designated V8-L and V8-S, of apparent MWs 27,000 and 12,000, respectively (Fig. 1a, b). In addition, a single 7-residue peptide (derived from the C-terminus of the intact protein) was released into the supernatant. The digested protein retained full spectral integrity, and its conformational stability in detergent solution allowed the V8-L and V8-S fragments to be delipidated and purified as a single complex by affinity chromatography on concanavalin A (Con-A)-Sephacrose¹¹. The two fragments were then resolved by gel filtration in organic solvents (Fig. 2). Figure 3 shows the complete amino acid sequence of the smaller (V8-S) fragment. This comprises the C-terminal third of the protein and contains the chromophore attachment site, the sites of light-induced phosphorylation, and one of the two -SH groups freely available for modification in the intact protein.

[15-³H]11-*cis*-retinal¹² was covalently linked to the protein by reductive fixation with KBH₄; the site of attachment is localized on the V8-S fragment (Fig. 1c). Sequence studies of ³H-labelled peptide 3 showed that radioactivity is released only at position 53, and is recovered in molar yield¹³. Sequence data on unlabelled protein confirmed the assignment of a lysine residue at this position, and this unambiguously identifies the chromophore binding site.

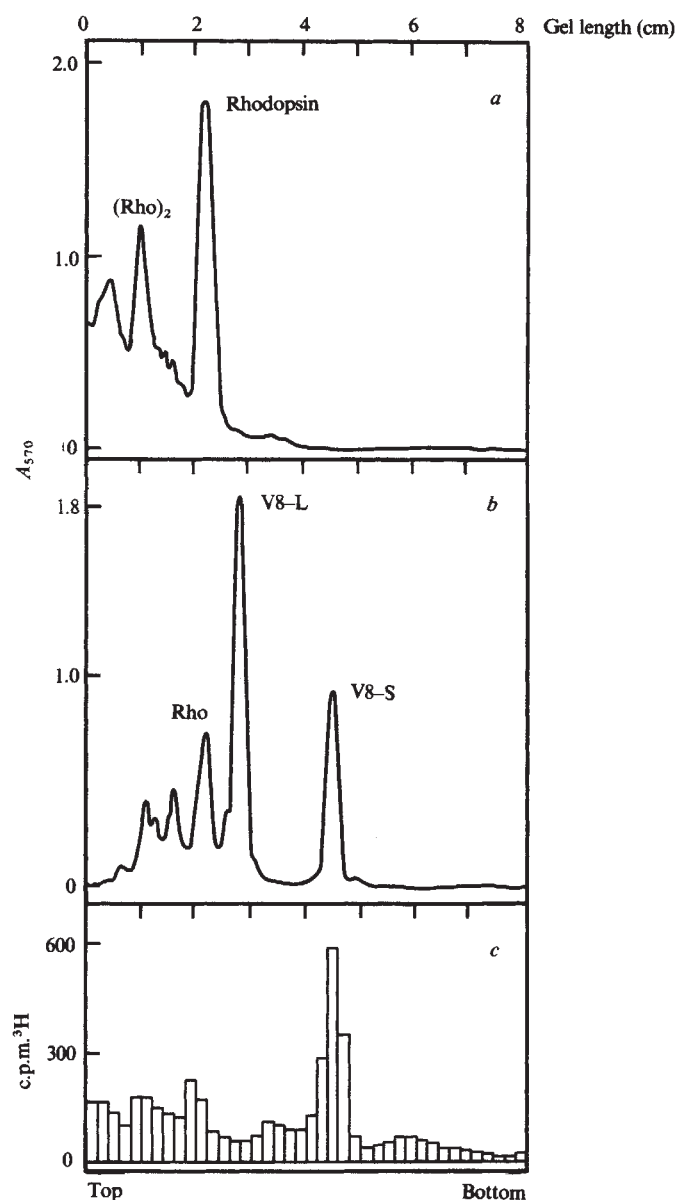


Fig. 1 Digestion of ovine rhodopsin *in situ*. Rod disk membranes were prepared from isolated sheep retina in dim red light as previously described²⁶. Purified photoreceptor membranes were suspended at a protein concentration of 2 mg ml⁻¹ in 67 mM sodium phosphate buffer pH 7.0, and digested for 3 h at 30 °C in the dark with 2% w/w protease V8 (*S. aureus*). Analysis of the digested material by SDS-urea polyacrylamide gel electrophoresis (10% w/w acrylamide, 8 M urea²⁷) revealed the presence of two fragments of apparent MWs 27,000 and 12,000, designated V8-L and V8-S, respectively (Fig. 1a, b). The supernatant contained a single peptide (peptide 6, Fig. 3) which was isolated by gel filtration on P4 in 5% v/v acetic acid. Bleached rhodopsin could be regenerated with [15-³H]11-*cis*-retinal¹³. Illumination of regenerated protein in the presence of 3 mg ml⁻¹ KBH₄ resulted in the specific labelling of the V8-S fragment (Fig. 1c).

After bleaching in the presence of a rod-cell kinase preparation and [γ -³²P]ATP, opsin is phosphorylated^{14,15}. In agreement with data from the bovine protein⁹, the residues containing ³²P were located in the region 6–15 and assigned to specific Ser and Thr residues¹⁵.

Native rhodopsin also contains two -SH groups which are freely available for alkylation^{16,17}; one of these is located in V8-L, the other is residue 33 on V8-S.

Comparison with partial sequence data from the bovine protein^{9,18} reveals almost complete homology between the two polypeptides. A conservative substitution has occurred at posi-

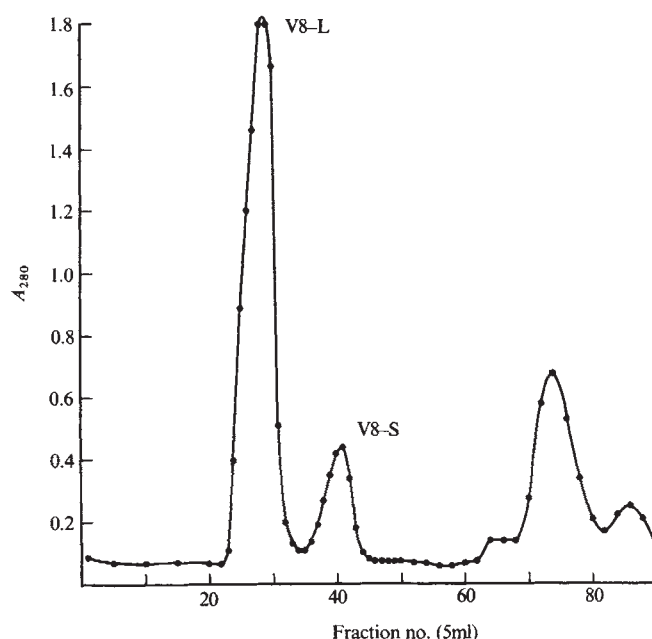


Fig. 2 Isolation of V8 proteolytic fragments. Proteolysed, washed disk membranes were solubilized in 3% w/v dodecyltrimethylammonium bromide (DTAB) and subjected to affinity chromatography on Con A-Sepharose 4B¹¹. The peak fraction of purified and delipidated V8-cleaved opsin contained 3–5 mg ml⁻¹ protein and had an A_{280}/A_{500} ratio of 1.65. The protein was dialysed against 0.2 M Tris-HCl pH 8.6, containing 0.25% w/v DTAB and 5 mM dithiothreitol, before carboxymethylation for 1 h in the dark with 10 mM iodoacetic acid. The two carboxymethylated fragments were then dialysed against distilled water and the lyophilized fragments separated on Sephadex LH60 equilibrated and eluted with 90% formic acid/ethanol 1:2 v/v.

tion 31, valine (bovine) replacing leucine. Pellicone *et al.*¹⁸ have inferred the presence of a tryptophan residue at 84 on the basis of chemical cleavage with 2-(2-nitrophenylsulphonyl)-3-methyl-3'-bromo indoline-skatole. We sometimes also find a phenylalanine at this position, but we do obtain specific cleavage at this point with the tryptophan-directed reagent, *o*-iodosobenzoic acid¹⁹. The remaining differences occur adjacent to the retinal-binding residue. The ovine sequence in this region is (54) Ala-Lys-Ser-Ser-Ser (50), which differs from the reported bovine sequence of -Ala-Lys-Thr-Ser-Ala- (ref. 18). In the case of the bovine protein, this region was first implicated as the retinal-binding site by Wang *et al.*²⁰ following their isolation of an Ala-retinyl lysine dipeptide from exhaustive pronase digestion of the whole protein, and its inferred location in the partial sequence data of Pellicone¹⁸. Considering the remarkable extent of homology in the rest of the compared sequences, it is surprising that this variability occurs in such a critical region of the molecule. In any event, the almost complete conservation of primary structure, particularly in the trans-membrane segments, is interesting. Whether this will turn out to reflect a feature of integral membrane proteins in general, or opsin in particular, remains to be seen.

Our other labelling procedures are incomplete, but it seems reasonable to assign residues 109 to ~96 as part of a substantial cytosolic loop sensitive to various proteases^{5-9,17}. Thereafter, the polypeptide chain probably traverses the bilayer, making a projected turn somewhere in the region of residues 72 to 63, before crossing the membrane once more to return to the cytosolic face at about residue 35. The remaining segment of the chain does not seem to re-enter the membrane. We predict the retinal-binding lysine to be within the bilayer, perhaps one third of the membrane width from the intradiskal surface. The clustering of hydroxyl groups immediately adjacent to this residue suggests that they may be involved in the protonation of the Schiff base linkage²¹.

109	108	107	106	105	104	103	102	101	100
S	A	T	T	Q	K	A	E	K	E
99	98	97	96	95	94	93	92	91	90
V	T	R	M	V	I	I	M	V	I
(1)			(2)						
89	88	87	86	85	84	83	82	81	80
A	F	L	I	C	W	L	P	Y	A
79	78	77	76	75	74	73	72	71	70
G	V	A	F	Y	I	F	T	H	Q
69	68	67	66	65	64	63	62	61	60
G	S	D	F	G	P	I	F	M	T
59	58	57	56	55	54	53	52	51	50
I	P	A	F	F	A	K	S	S	S
49	48	47	46	45	44	43	42	41	40
V	Y	N	P	V	I	Y	I	M	M
			(3)						
39	38	37	36	35	34	33	32	31	30
N	K	Q	F	R	N	C	M	L	T
			(4)						
29	28	27	26	25	24	23	22	21	20
T	L	C	C	G	K	N	P	L	G
			(5)						
19	18	17	16	15	14	13	12	11	10
D	D	E	A	S	T	T	V	S	K
9	8	7	6	5	4	3	2	1	
T	E	T	S	Q	(6) V	A	P	A	- C

Fig. 3 Complete amino acid sequence of the C-terminal V8-S fragment of ovine rhodopsin. Purified V8-S was subjected to cleavage with a 100-fold molar excess of CNBr in 70% formic acid for 24 h at room temperature in the dark. The fragments were purified using Sephadex LH60 and LH20 in 90% formic acid/ethanol 1:2 v/v. Peptides 1–4 were coupled to aminoethyl-aminopropyl (AEAP) glass by the C-terminal homoserine lactone²⁸; peptide 5 was coupled to AEAP glass following *p*-phenylene diisothiocyanate activation²⁹. These were sequenced using the Rank-Hilger solid phase APS 240 sequencer, whereas peptide 6 was sequenced manually³⁰. The fragments were aligned as follows: peptide 1 from its amino-terminal identity (Ser) with V8-S; peptides 2 and 3 from the structure of an overlapping peptide obtained on digestion of V8-S with *o*-iodosobenzoic acid in 80% aqueous acetic acid, 4 M guanidinium chloride¹⁹; peptides 4 and 5 from the C-terminal V8-sensitive glutamic acid residue on peptide 5, and peptide 6 from the lack of a methionine or a C-terminal acidic residue¹⁷. When [15-³H]11-*cis*-retinal was covalently fixed to the protein, peptide 3 was the only labelled fragment¹³.

Note that several glutamic acid residues (102, 100 and 17) do not act as substrates for protease V8, and that certain -SH groups (26 and 27) cannot be alkylated in the intact protein, all of which may represent clues to the tertiary structure of rhodopsin.

An analogous light-sensitive protein, bacteriorhodopsin, is present in the membrane of *Halobacterium halobium*²². Although they may have some features of general molecular morphology in common, the data available for the two vertebrate opsins indicate no obvious homology. This is particularly true of the residues surrounding the retinal attachment sites^{23–25}, and the relative position of that site in the whole structure.

However, further conformational studies may reveal structural similarities in these regions.

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Note added in proof: The site of attachment of retinal in bacteriorhodopsin has been reassigned to lysine 216 (ref. 31). This C-terminal location is more analogous to the structural arrangement in ovine rhodopsin.

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Gated binding of ligands to proteins

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The binding of ligands to proteins often occurs at rates that approach the diffusion-controlled limit¹. For spherical molecules with negligible interactions apart from isotropic reactivity on contact, the diffusion-controlled rate constant is $k_D \equiv 4\pi RD$, where R is the distance between molecular centres at contact and D is the relative translational diffusion constant^{1–3}. Deviations from this limiting rate due to interaction potentials^{4,5}, hydrodynamic interactions^{6,7} and static geometric features of the binding sites^{8–13} have previously received much attention. Here, we point out another possible cause of such deviations, the dynamic modulation of binding site accessibility due to internal motions of the protein. Such motions may occur, for example, in proteins such as lysozyme, in which the active site cleft opens and closes in a stochastic manner^{14,15}.

Consider the simplest case of spherical molecules with negligible interactions apart from isotropic reactivity on contact, and suppose that the protein reactivity is modulated by an internal gate that requires negligible time to open or close. Let $h(t)$ be a

characteristic function describing the dynamics of the gate; $h(t) = 1$ at times when the gate is open so that the protein is reactive, and $h(t) = 0$ otherwise. The average binding rate, which would be observed in a typical kinetics experiment, is

$$\frac{dC_P}{dt} = -kC_PC_L^0 \quad (1)$$

where $C_P(t)$ is the concentration in cm^{-3} of unliganded protein (assumed infinitely dilute) and C_L^0 is the concentration of ligand ($C_L^0 \gg C_P$). The rate constant k is given by

$$k = \langle 4\pi R^2 k_s h(t) \rho(R, t) \rangle \quad (2)$$

where k_s is the specific rate constant when the gate is open ($4\pi R^2 k_s$ is identical to the rate constant k in Noyes' review²), $\rho(r, t)C_L^0$ is the concentration of ligand at a distance r from an unliganded protein at time t (corresponding to the concentration c_r in Noyes' review²) and the brackets indicate a time average. The density $\rho(r, t)$ is governed by the equation for diffusion to a sink at $r = R$,

$$\frac{\partial \rho}{\partial t} = r^{-2} \frac{\partial}{\partial r} \left(r^2 D \frac{\partial \rho}{\partial r} \right) - k_s h(t) \rho \delta(r - R) \quad (3)$$

with a reflecting wall boundary condition at $r = R$; the normalization condition is that $\rho = 1$ at equilibrium, when the gates have been closed at all past times. If the gates are fixed in the open state, one obtains from equation (2) the familiar steady-state results² $k = k_{eq} = 4\pi R^2 k_s$, if $k_D \gg k_{eq}$; $k = k_D$ if $k_{eq} \gg k_D$; and $k = k_{eq} = k_{eq} k_D (k_{eq} + k_D)^{-1}$ in general.

Now suppose that the internal dynamics of the proteins are such that when the gates open they remain open for the fixed but arbitrary time τ_0 , and when they close remain closed for times that are long compared with the diffusional relaxation time $\tau_d = R^2/D$, so that ρ relaxes to the equilibrium density. Solution of equation (2) for this case yields $k = K(\tau_0) \langle h(t) \rangle$ where

$$K(\tau_0) = k_{\infty} + k_{\infty}^2 k_D^{-1} \tau_d^{1/2} \{ 2(\pi \tau_0)^{-1/2} - (\beta \tau_0)^{-1} [1 - \exp(\beta^2 \tau_0) \text{erfc}(\beta \tau_0^{1/2})] \} \quad (4)$$

and $\beta \equiv k_D^{-1} \tau_d^{-1/2} (k_{eq} + k_D)$. The rate constant in this case has a simple physical interpretation; it is just the product of fraction of the time that the gate is open, $\langle h(t) \rangle$, and the average rate constant, $K(\tau_0)$, for ligand binding during a period τ_0 starting with an equilibrium density $\rho = 1$. In the limit that $\tau_0 \ll \tau_d$, the rate constant becomes $k = k_{eq} \langle h(t) \rangle$; in the limit that $\tau_0 \gg \tau_d$, it becomes $k = k_{\infty} \langle h(t) \rangle$. The last equation holds for any lifetime of the closed gate state.

Situations in which the various cases described above would be approximately realized can be identified by considering the variation of the protein energy $E(\xi)$ as a function of the gate coordinate ξ (compare with the hinge bending energy for lysozyme¹⁴). For example, the case with closed gate lifetimes $\gg \tau_d$ and $\tau_0 \ll \tau_d$ could occur if $E(\xi)$ were parabolic with the minimum energy $E = 0$ corresponding to a closed gate and energies $> k_B T$ for the open states ($k_B T$ is the product of the Boltzmann constant and the absolute temperature). The case with closed gate lifetimes $\gg \tau_d$ and $\tau_0 \gg \tau_d$ could occur if $E(\xi)$ had a barrier separating stable open and closed states.

Detailed application of these ideas to particular hinge-bending proteins¹⁶ or other systems (for example, dynamically modulated channels in proteins¹⁷ or membranes¹⁸) will require generalization of the present results to allow for variable lifetimes of the states, anisotropic surface reactivity and other features of real systems. Full derivations of the present and generalized results will be presented elsewhere.

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Low-frequency resonance Raman spectroscopy of the deoxyhaemoglobin transient of photolysed carboxyhaemoglobin

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Among other methods¹, resonance Raman spectroscopy is a particularly useful technique for the study of haem proteins because it monitors conformational changes that are reflected in vibrational degrees of freedom of the haem moiety. Its application to haemoglobin (Hb)^{2–7} has recently been extended by the development of time-resolved techniques. High frequency bands in time-resolved resonance Raman (TR³) spectra obtained with excitation into the α, β absorption bands have already been used to study the conformational changes of the haem associated with the photolytic removal of CO from carboxyhaemoglobin (HbCO) at 30 ps and 20 ns (ref. 7). We report here TR³ spectra, obtained by excitation into the Soret absorption band, containing low-frequency bands which provide a detailed characterization of the Hb conformation formed within 8 ns of the photolysis of HbCO.

TR³ spectra in the 100–450 cm^{-1} region were generated by 442 nm radiation (3 mJ per pulse) from a dye laser pumped by the third harmonic frequency of a Nd:YAG laser (Quanta Ray DCR and PDL system) operating at 10 Hz. Samples of stable deoxyHb were prepared by either extended flushing of HbO₂ with N₂ or by addition of a twofold excess of dithionite. HbCO was prepared by flushing deoxyHb samples with CO and sealing the sample under 1 atm CO. The samples (50 μM in haem) were studied in a cell spinning at 1,800 r.p.m. Raman scattering was collected by an $f/1.2$ lens and focused through a 1-m spectrograph equipped with an 1,800 groove mm^{-1} holographically ruled grating and on to a doubly intensified Vidicon camera (EG & G/PAR, model ISIT). Typical TR³ spectra were obtained with 400- μm slits (10 cm^{-1} s.b.p.) and consisted of an accumulation of 5,000 separate laser exposures. Neon and krypton emission lines were used to determine the absolute positions of the laser line and Raman bands from which the frequency shifts were calculated.

Previous workers^{7,8} have observed that the transient species observed in the TR³ spectra near 1,500 cm^{-1} resembles stable deoxyHb, but that small frequency differences (1–6 cm^{-1}) between the transient and stable deoxyHb suggest that the haem core is slightly larger than in deoxyHb. It is thought that these frequency differences may be associated with the transition of

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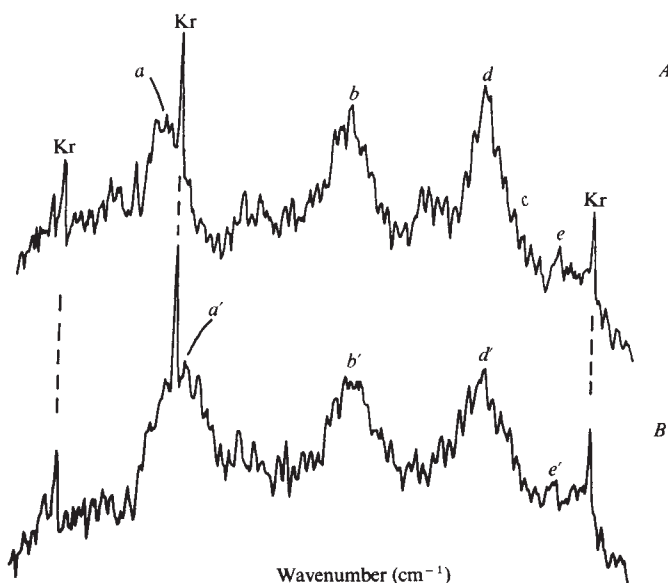


Fig. 1 Low-frequency (100–450 cm^{-1}) region of the resonance Raman spectrum of deoxyHb formed chemically by the addition of dithionite (A) and deoxyHb formed within 8 ns of the 442 nm photolysis of HbCO (B). Frequency scales of both spectra are calibrated by krypton lines which were optically superimposed. Frequency positions of resonance Raman bands are given in Table 1.

deoxyHb from the high oxygen affinity (R) state to the low oxygen affinity (T) state.

The low-frequency spectra allow the transient to be characterized in more detail. The low-frequency (100–450 cm^{-1}) TR^3 spectra obtained with pulsed (8 ns) excitation of the deoxyHb transient formed immediately after the removal of CO from HbCO is shown in Fig. 1 and the frequency positions of these bands are given in Table 1. The appearance of the $225 \pm 2 \text{ cm}^{-1}$ band in the TR^3 spectrum represents a shift of 11 cm^{-1} from the $214 \pm 2 \text{ cm}^{-1}$ band seen in the pulsed resonance Raman spectrum of chemically stabilized deoxyHb (Table 1). The positions of these bands correlate well with the frequencies observed for the R (221 cm^{-1}) and T (215 cm^{-1}) forms of chemically stabilized deoxyHb by Kitagawa and co-workers⁶.

Other aspects of the low-frequency TR^3 spectrum caution against an exact identification of the transient species with chemically stabilized (R)-deoxyHb: (1) the $225 \pm 2 \text{ cm}^{-1}$ band lies about 4 cm^{-1} higher in frequency than the 221 cm^{-1} observed in chemically stabilized (R)-deoxyHb. (2) The 303 cm^{-1} band which has the same frequency in chemically stabilized

samples of (R)- and (T)-deoxyHb appears at $306 \pm \text{cm}^{-1}$ in the TR^3 spectrum. (3) The 341 cm^{-1} band, although a weak feature in both the (R)- and (T)-deoxyHb spectrum, is not seen at all in the TR^3 spectrum. (4) A general broadening of the low-frequency TR^3 bands is observed.

These differences may be attributable to any one, or to a combination of three factors. First, the resonance Raman spectra of (R)- and (T)-deoxyHb have been recorded only for chemically stabilized forms, the preparation of which involves chemical modification of the protein. As a result, these chemically formed models of (R)- and (T)-deoxyHb may not be exact representations of the deoxyHb formed photolytically. Second, the photolytically generated (R)-deoxyHb may be a modified form of the chemically stabilized (R)-deoxyHb to which it is converted at times longer than 8 ns. TR^3 spectra over longer reaction times should measure such a conformational transformation. Third, the TR^3 spectrum may contain contributions from excited electronic states of deoxyHb. The TR^3 spectra of excited electronic states exhibit both frequency shifts and line broadening relative to the ground electronic state and have been observed in many different molecular systems in similar experimental conditions to those used here^{9–16}. The importance of excited electronic states to transient absorption spectra measured in the HbCO system has been suggested previously¹⁷.

Qualitatively, the spectra suggest that the transient resembles (R)-deoxyHb. The band in the 220 cm^{-1} region has been correlated with the $\text{Fe(II)}-\text{N}_\epsilon$ (His F8) bond⁶ and the high frequency of this band in the spectrum of the transient is evidence for a compressed $\text{Fe(II)}-\text{N}_\epsilon$ (His F8) bond. This would arise from either an expansion of the Fe electron cloud and/or movement of the iron towards the histidine in times less than 8 ns, with movement of the histidine-containing protein chain occurring on a longer time scale.

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Table 1 Frequency positions (cm^{-1}) of resonance Raman bands in the pulsed resonance Raman (PRR), c.w. and time-resolved resonance Raman (TR^3) spectra of deoxyhaemoglobin

Band	Hb† PRR‡	(T)-Hb§ c.w.	Band*	HbCo TR^3	(R)-Hb§ c.w.
a	214 ± 2	215	a'	225 ± 2	221
b	301 ± 2	302	b'	306 ± 2	302
c	340 ± 2	341	—	—	341
d	364 ± 1	366	d'	365 ± 1	366
e	403 ± 2	404	e'	403 ± 2	404

Deoxyhaemoglobin was prepared either in a chemically stable form or by photolysis of HbCO. The frequency of laser excitation (c.w. and pulsed) was 442 nm. Samples were between 0.05 mM (this work) and (1 mM (ref. 6)) in haem and were examined at room temperature.

*See Fig. 1.

†Chemically stabilized by addition of dithionite (see text and Fig. 1a).

‡This work.

§Chemically stabilized⁶.

||Prepared by N_2 purging and examined under an atmosphere of CO.

Corrigenda

In the letter 'Stringency without ppGpp accumulation' by A. Spodaro *et al.*, *Nature* **291**, 256–258, line 5 in paragraph 6 should read 'present in TA1001 is caused by the *hisT* mutation, although it is possible that strain TA1001 harbours one or more additional mutations'.

In the matters arising item 'Expression of herpesvirus-induced antigens in human cervical cancer' by C. C. Smith *et al.*, *Nature* **292**, 388–389, the authors in ref. 13 should read 'Smith, C. C., Aurelian, L., Cohen, G. H. & Eisenberg, R.'.

In the letter 'Thermoproteales—a third order of thermoacidophilic archaeobacteria' by W. Zillig *et al.*, *Nature* **293**, 85–86, the journal cited in refs 17 and 20 should read 'Zentbl. Bakt. Hyg., I. Abt. Orig. Cl.' and the journal in ref. 21 is C2 of that series. The volume number for ref. 25 should read 41.

BOOK REVIEWS

Polarized views of Quaternary ice sheets

D.Q. Bowen

DURING the past decade the major thrust in Quaternary research has been directed towards an investigation of oceanic deposits, and from such work a definitive stratigraphic framework for the entire period has emerged. At the same time, attempts have been made to correlate continental events with this unique actualistic yardstick. Hitherto only blessed with varying degrees of success, the outstanding exception has been the CLIMAP reconstruction of the Earth's surface 18,000 years ago. An essential element in this was an estimation of the extent and dimensions of the ice-sheets — an input made by some of the contributors to *The Last Great Ice Sheets*. But although the original aim was to reconstruct the ice-sheets in three dimensions as a prelude to numerical modelling of the contemporary atmosphere, this had to be modified due to progressive realization that the extent and palaeogeology of those ice-sheets was imperfectly known.

In this handsomely produced collection of essays, complete in a case with folding maps, we have an attempt at understanding the basic parameters of the ice-sheets, divided into three general sections: Bjorn Andersen and others discuss the timing and maximum extent of ice-sheets and valley glaciers; Hollin, Schilling and Hughes employ numerical models for estimating ice-sheet and valley glacier elevations; and Denton, Hughes, Stuiver and others attempt reconstructions of ice extent and dimension between 21,000 and 17,000 years ago.

Before any such reconstruction can be made, the maximum extent and flow lines of the ice must be established. Unfortunately good stratigraphic control is rarely available, geomorphic features are often equivocal and too often the sole arbiter for control is radiocarbon dating — frequently of organic materials incorporated into glacial deposits. Furthermore, virtually no dates exist for the critical period between 35,000 and 12,000 years ago on the northern margins of the ice-sheets. Together, these factors have engendered an uncertainty, which has led to a major divergence of opinion as to the extent and timing of the maximum ice-cover in the far north.

What can be called the "minimum extent school" of thought maintains that northern and southern limits of the ice-sheets were out of phase: the southern maximum being attained 21,000 to 17,000 years ago, the northern maximum during

The Last Great Ice Sheets. Edited by George H. Denton and Terence J. Hughes. Pp.484 plus maps. ISBN 0-471-06006-2. (Wiley: 1981.) £70.20, \$126.35.

the late-Glacial or even during the early post-Glacial, a lack of synchronism attributed to a late availability of precipitation as the oceanic polar front migrated northwards at the end of the glacial stage. On the other hand, the "maximum extent school", heavily hall-marked in this book, argue that both northern and southern limits were synchronous and that a unified northern ice-complex behaved as a single dynamic entity. This view derives from Antarctic perspectives, notably from Denton and Hughes, the editors of this volume, who have worked extensively in the far south. It could, of course, be argued that theirs is a proper uniformitarian view. Yet, the Antarctic ice-sheet is polar, whereas ice-sheets of the Northern Hemisphere were primarily mid- and higher-latitude ones, for which, moreover, no analogue exists today. Furthermore, the Antarctic model relates very largely to the unstable West Antarctic ice-sheet with its fringe of floating ice-shelves.

The inferred sequence presented in the book is both novel and challenging: grounded ice-sheets would form on shallow continental shelves and inland Arctic seas, especially when surrounded by protective islands as in the Kara and Barents Seas and Queen Elizabeth Islands. Rapid ice-thickening would create ice-domes, the nuclei of marine ice-sheets. Beyond these, ice-shelves would buttress the inherently unstable ice-domes, and would link the North American and Eurasian Arctic ice masses. During deglaciation a rising sea-level would free ice-shelves from bedrock underpinning points. Because they would no longer buttress the ice-domes, collapse of the domes by downdrawing followed, glacier surging occurring along troughs leading to calving bays.

But is the Antarctic perspective applicable to the Northern Hemisphere? Did marine ice-sheets actually exist? Not according to those who work in the Canadian Arctic and Spitsbergen who are intractably opposed to these views. And here it must be said that Western geologists have not had sufficient opportunity to examine cores from the Kara and Barents Seas, pronounced to be glacial deposits by their Soviet counterparts: they could yet turn out to be glacio-marine and not

glacial. It could also be argued that ice-sheets as large and thick as those required by the "maximum extent school" would not have responded as swiftly to climatic changes as a now increasing volume of geological evidence shows they did.

The Antarctic paradigm for northern ice-sheets is topical, innovative and above all a major challenge. It may already have some relevance in areas such as the coast of Maine, or possibly the Irish Sea. But the outlook is one of intensified debate between the two schools of thought, with the burden of proof leaning on those who subscribe to what even the editors themselves call an "outrageous hypothesis". □

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Secrets of secretion

B.L. Ginsborg

The Electrophysiology of Gland Cells. Monographs of the Physiological Society, No.36. By O.H. Petersen. Pp.253. ISBN 0-12-553120-6. (Academic: 1981.) £20.40, \$49.

UNCHARITABLE proponents of the "important question" approach to research are apt to point out that electrical activity is not the function of gland cells. One might retort that neither is it the function of motor axons nor of muscle fibres, but this has not prevented electrical studies providing major insights into the motor system. That line of defence is, however, unnecessary for this admirable monograph which has a title more restrictive than its subject matter. True, it records and analyses the substantial efforts that have been made over the past ten years or so to understand the electrical properties and activity of gland cells, but at every turn an attempt is made to relate electrical to secretory activity. The illustrations are not confined to circuit diagrams and records of voltage and current, but include records of flow and of ion concentrations, and electron micrographs.

The diversity of approach is matched by the diversity of material: pancreas, liver, adrenal medulla and pituitary of

mammals, and salivary glands of mammals, insects and molluscs. For good measure there are also at least mentions of the adrenal cortex, the thyroid and the parathyroid. All this is within the compass of a mere 250 pages, achieved not by omission but by virtue of the author's enviably direct style.

The approach wherever possible is quantitative and starts from the premise that things may not be nearly so complicated as they might seem at first sight. "It would be easy to dwell on the great diversity of electrophysiological patterns observed in different gland cells, but it may be more profitable to point out the many similarities" is how the last, integrative, chapter begins. It is one of the few asides the author permits himself, and it conveys the spirit which informs most of the preceding text: above all, no mystification.

In so far as electrophysiology in its strict sense is concerned, the author's message is the following. In cells which are not "excitable", mainly vertebrate and insect exocrine cells, changes in membrane potential are contingent on, rather than necessary to secretion. For the experimentalist they serve the useful purpose of signalling the increase in ionic conductance which is essential to load the cell through the basal membrane. Recent investigations, particularly those of the author and his colleague Iwatsuki, have provided strong support for the idea that the

conductance changes brought about by secretagogues are secondary to an increase in cytosolic calcium. On the other hand, in vertebrate endocrine cells and some molluscan exocrine cells, which are excitable, action potentials may perform an essential role in opening voltage-sensitive ion channels, particularly to calcium. The evidence bearing on these hypotheses is assembled with great economy, missing links are underlined and for the faint-hearted each of the 14 chapters has a short, lucid summary.

The electrophysiology of secretion is deemed to have been discovered in 1857 by de Bois Reymond. Since then, until recently, progress was unremarkable. Schäfer's textbook of physiology of 1898

devotes about a page to the subject, pride of place being given to the work of Bayliss and Bradford on the salivary gland. More than 50 years later in the eleventh edition of Starling's textbook of physiology the subject rates only a short paragraph and the same papers are cited. Ten years later the thirteenth edition has no trace of the subject. If this monograph does not persuade teachers of physiology to pay some attention to current work on secretion, nothing will. For those who work in the field, it need hardly be said that the book is indispensable. □

B.L. Ginsborg is a Professor in the Department of Pharmacology at the University of Edinburgh.

The questions of what and where to eat

N.B. Davies

Foraging Behavior: Ecological, Ethological and Psychological Approaches. Edited by Alan C. Kamil and Theodore D. Sargent. Pp.534. ISBN 0-8240-7068-2. (Garland STPM Press: 1981.) \$52.50.

ALL predators, whether lions chasing gazelle on the African plains or birds looking for berries in a back garden, must make decisions — where to hunt, which prey to select, when to move on to a new feeding area and so on. Ethologists have been interested in the costs and benefits associated with these decisions and how they influence survival and reproductive success, while psychologists have been more concerned with the mechanisms that underlie the behaviour, for example motivational and learning rules. Based on an Animal Behaviour Society meeting in Seattle, Washington, this collection of essays is a stimulating summary of the recent advances in these approaches.

The book is divided into four sections, each with an introduction by the editors. The first considers optimal foraging theory which is an attempt to predict which particular trade-off between costs and benefits will give the maximum net benefit to the individual. Krebs, Houston and Charnov give a good review of the models and show the generality of simple decision rules. Subsequent chapters show that the same model can be used to predict how long a bee (Pyke, Waddington, Heinrich) or hummingbird (Gass, Montgomerie) should remain at a flower before moving on to the next one, how many prey a parent bird should carry back to the nest (Orians) and for how long a male dungfly should copulate, because all of these decisions involve the exploitation of a depleting resource.

In the second section, several field studies emphasize the complexity of

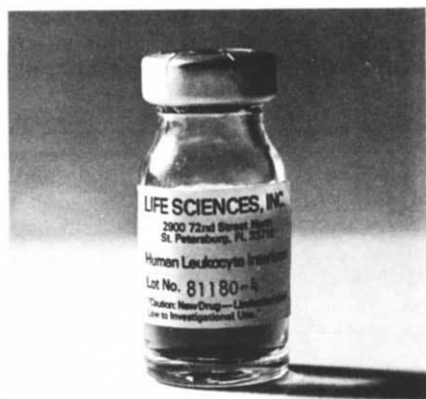
foraging behaviour in nature. For example, it is clear that predictions based solely on energy maximization do not describe prey choice in protozoa (Rapport), wading birds (Goss-Custard) or monkeys (Glander) because all need a critical balance of nutrients in their diet. Another complication, shown clearly in the chapter on sanderling territories by Myers, Connors and Pitelka, is that decision rules may be determined by long-term advantage rather than immediate gain.

Predators presumably do not work out mathematical equations to decide how to forage, and Section 3 examines some of the simple rules they may use to achieve the optimal behaviour. Pulliam shows that a learning rule, known as the "matching law", derived from psychological experiments, makes good functional sense as it maximizes the rate of food intake. Pietrewicz and Kamil describe some elegant experiments on how birds recognize moths and relate their findings to the evolution of crypsis by the prey (Sargent). The final section on the genetic basis of foraging behaviour includes an excellent chapter by Arnold on prey recognition by garter snakes.

Several authors, Zach and Smith, for example, suggest that nature is much too complex for simple models to be of any use. This seems an unnecessarily pessimistic point of view. Not only does the rejection of simple models often lead to a better understanding of behaviour, but it is also possible that what looks like very complicated behaviour will after all turn out to involve simple decisions once the field worker has understood properly the natural history of his animal and its environment. □

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Almost everything you wanted to know about chromosomes

R.A. Laskey

Eucaryotic Chromosomes. Gene Expression, Vol.2, 2nd Edn. By Benjamin Lewin. Pp.1,160. ISBN pbk 0-471-01977-1; ISBN hbk 0-471-01976-3. (Wiley: 1981.) Hbk £33.70, \$60.65; pbk £13.25, \$29.

THE AMOUNT of literature on cell biology is exploding at a frightening rate, so do we need this massive book? Not only is the answer yes, but I believe that this is the most valuable block of eleven hundred pages in the current cell biology literature. A vast amount of recent information has been selected, digested and evaluated to produce a unique analysis of this rapidly expanding field.

The contents provide an accurate account of research on the eukaryotic chromosome up to the end of 1979. New discoveries in the six years between the first and second editions included the nucleosome subunits of chromatin and intervening sequences interrupting genes. These alone would justify a second edition, but a mass of detailed information on genome structure and organization has also emerged as a result of advances in recombinant DNA technology and DNA sequencing techniques. Therefore, this new edition is not just a minor revision but essentially a new book, being over twice the length of its predecessor.

The first part deals with cell structure and genetics. Surprisingly for a book entitled *Eucaryotic Chromosomes*, the first 70 pages provide a discussion of the cytoskeleton. However this unexpected bonus is a good introduction to the mitotic and meiotic apparatus, which are also discussed in this part together with the cell cycle and cell fusion studies. Part 2 deals with the organization of the genetic apparatus, concentrating on structure of chromatin and chromosomes. Part 3 covers genome organization, and here there is a sad reflection of the state of work on eukaryotic DNA replication which takes up only 13 pages, while in contrast the next chapter on organelle genomes occupies 55 pages. The final part is an account of the expression of genetic information and this section provides frequent illustrations of the book's principal strength, namely that it is not just a catalogue, but that it provides a level of critical discussion expected of a specialist review. Sustaining this penetrating standard of analysis for more than 900 pages of text is a remarkable achievement. The cautious judgements of controversial issues will ensure that the book remains valuable for many years, even though the subject matter continues to evolve rapidly. Unfortunately, however, the continued caution and criticism sometimes distract from the main thrust of the book.

The problem of extracting principal messages and key concepts from such a

detailed discussion could be overcome by providing summary or conclusion sections. The final few pages of the book go some way towards this objective, but I feel that a work of this size would benefit greatly from a summary of the key points from each chapter. Because it is difficult to obtain clear overviews of the subject from the book, I would not recommend it for a novice student whose interest in the field has not been aroused already; its scope and scale could be simply overwhelming. I believe the most serious fault of the book is this lack of contrast. Apart from the addition of summaries, a more frequent use of diagrams to illustrate key concepts might also improve the emphasis. That said, the diagrams which are provided are effective, as are the tables which condense large amounts of information into a readily digestible form. The few photographs are also well chosen to provide appropriate illustration of structures.

Perhaps the most striking demonstration of the scope of this remarkable book is the 169 pages of references. Almost 4,000 papers are cited, making this an unusually

valuable bibliographical source. Despite the large number of references cited they cause surprisingly little disruption to the text which remains fluent. Even more extraordinary is the fact that this is only one of the three volumes which comprise *Gene Expression* by this author; the other two deal with bacterial genomes (Vol.1) and plasmids and phages (Vol.3).

A potential flaw in a book which attempts such detailed coverage of such a wide field is factual error. However the author has maintained a high level of accuracy. The only faults of this nature I could find while reading the entire book were minor errors of detail rather than those of major facts or principles.

Although I would not recommend this book as an introduction to the subject, I feel that it is simply the best specialized discussion of the field that I have seen and I would recommend it enthusiastically to advanced students, teachers and researchers. □

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Theories of conduction in organic solids

Martin R. Willis

Electrical Transport in Solids. By Kwan C. Kao and Wei Hwang. Pp.663. ISBN 0-08-023973-0. (Pergamon: 1981.) £50, \$120.

ORGANIC solids exhibit the full range of electrical behaviour; they may be excellent insulators or superconductors. The polycyclic aromatic hydrocarbons, of which anthracene is typical, have been the most fully characterized and extensively studied, and are possibly the most fully understood. These materials are high-gap semiconductors or insulators, and band theory has been widely used for the interpretation of results. But since its applicability to materials with such narrow bands and low mobilities is questionable, other approaches such as hopping, tunnelling and polaron conduction have been applied.

The authors of this book have performed a valuable service by bringing together in a coherent form many of the theories applicable to conduction in organic solids, particularly polycyclic hydrocarbons. Much of the theory, particularly band theory, trapping, carrier injection and space charge limited currents, was originally derived for inorganic systems, but the limitations in its application to organic solids are stressed and numerous experimental results are quoted for such materials. Other theories,

such as aspects of the exciton theory of photo-carrier generation were developed specially for molecular crystals. For those interested in solar energy conversion there is a section on photo-voltaic effects. The coverage is even greater than appears from the index, for Seebeck and Peltier effects are not listed but are well covered in the text.

A particularly helpful feature of the book is the presentation of such diverse theories using a common set of symbols, clearly defined at the beginning of the text, and mainly in SI units. Experimental results are, however, quoted as originally published and hence are mainly in egs units. Energies are in electron volts, in line with current practice, but it is confusing that activation energy for conduction is defined in such a way as to make it equal to the band gap of an intrinsic semiconductor, in preference to the usual definition based on the Arrhenius equation.

Taken overall, this is an extremely welcome addition to the literature of the organic solid state and there will be few workers in the field who will not want a copy on their shelves, or at least in the library of their institution. □

Martin R. Willis is Reader in Physical Chemistry at the University of Nottingham.

BOOKS RECEIVED

Astronomy

- BRECHER, K. and FEIRTAG, M. (eds). *Astronomy of the Ancients*. Pp.206. Hbk ISBN 0-262-02137-4; pbk ISBN 0-262-52070-2. (MIT Press: 1981.) Hbk np; pbk \$7.95.
- ROWAN-ROBINSON, M. *Cosmology*. 2nd Edn. Oxford Physics Series, Vol.15. Pp.152. Hbk ISBN 0-19-851857-9; pbk ISBN 0-19-851858-7. (Clarendon Press/Oxford University Press: 1981.) Hbk £12.50; pbk £5.95.
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Biological Sciences

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General

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- ARIOTTI, P.E. and BRONOWSKI, R. (eds). *The Visionary Eye. Essays in the Arts, Literature and Science*. J. Bronowski. Pp.185. Pbk ISBN 0-262-02129-3. (MIT Press: 1981.) Pbk \$4.95.
- ATKINSON, G. and ZUCKERMAN, J.J. (eds). *Origin and Chemistry of Petroleum*. Proceedings of the 3rd Annual Karcher Symposium, Oklahoma, May 1979. Pp.120. ISBN 0-08-126179-5. (Pergamon: 1981.) £10, \$24.
- AUSTRALIAN ACADEMY OF SCIENCE. *Liquid Fuels: What Can Australia Do? Papers presented to a meeting of the Science and Industry Forum of the Australian Academy of Science October 1980*. Pp.109. Pbk ISBN 0-85847-087-X. (Australian Academy of Science, Canberra: 1981.) Pbk \$5.95.
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- BAYNES, K. and PUGH, F. *The Art of the Engineer*. Pp.240. ISBN 0-7188-2506-3. (Lutterworth Press, Guildford, Surrey: 1981.) £25 before 1.9.81, £28 after 1.9.81.

ANNOUNCEMENTS

Awards

The Royal Society has awarded the three Royal Medals for 1981 to: **Sir Geoffrey Wilkinson**, for his contributions to preparative inorganic chemistry and in particular to the synthesis and application of organometallic compounds; **Dr Marthe L. Vogt**, for her contributions to synaptic biochemistry and pharmacology and to laying the basis of modern neuropharmacology; **Dr R. Riley**, for his contributions to understanding the genetics of wheat and the development of new methods of producing improved varieties.

The Council of the Royal Society has awarded the Esso Energy Award for 1981 to **Dr B. Linnhoff** for his approach to the design of heat exchanger networks used in industry, leading to substantial savings in energy use.

Dr Eric J. Chaisson (Harvard University and Harvard-Smithsonian Center for Astrophysics in Cambridge, Massachusetts), is the 1981 winner of the American Institute of Physics-United States Steel Foundation Science-Writing Award for his book, *Cosmic Dawn: The Origins of Matter and Life*.

The Association for Radiation Research has presented Weiss Medals to **Dr E.J. Land** (Paterson Laboratories, Christie Hospital) and **Dr A.J. Swallow** (Holt Radium Institute, Manchester) for their work in the application of fast-reaction techniques in radiation science, especially in areas of biological interest.

The Marconi International Fellowship is awarded to a man or woman whose career is characterized by originality in science and technology and by concern for human welfare. Nominations from universities, industries, learned societies and academies of all countries, as well as from individuals in science, industry and public life should be submitted by 15 April 1982 for the Ninth Marconi Fellowship to: Marconi International Fellowship Council, Aspen Institute for Humanistic Studies, 1229 University Avenue, Boulder, Colorado 80302, USA.

The Carbohydrate Chemistry Award sponsored by Tate and Lyle Ltd will be made to the member of the Royal Society of Chemistry who shall have published during 1980 and in the preceding five years, the most meritorious contributions to the knowledge of any aspect of carbohydrate chemistry. The recipient must be under 36 years of age and a citizen of the United Kingdom; the major part of the relevant scientific contributions must have been carried out and published in the United Kingdom. Applications to S.S. Langer, The Royal Society of Chemistry, Burlington House, London W1, UK by 31 October 1981.

The Harrison Memorial Prize will be awarded to the chemist, a natural-born British subject under 30 years of age, who in the five years ending 1 December 1981, has conducted the most meritorious promising original investigations in chemistry and published the results of those investigations in a scientific periodical or periodicals. Applications to the Local Activities Officer of the Royal Society of Chemistry, Burlington House, Piccadilly, London W1, UK by 31 December 1981.

The Corday-Morgan Medal and Prize will be made in respect of the year 1980 to chemists of British nationality under the age of 37 who have published during 1980 and in the preceding five years, the most meritorious contributions to experimental chemistry in their respective fields. Applications by 31 December 1981 to The Royal Society of Chemistry, Burlington House, London W1, UK.

If you work in a university outside the UK, or in a polytechnic, college or school anywhere and your work is held up for lack of modest funding, the Research Fund of the Royal Society of Chemistry may be able to help you. Applications to Mrs E.S. Wellingham, The Royal Society of Chemistry, Burlington House, London W1, UK by 1 November 1981.

Appointments

Professor J.I.G. Cadogan, chief scientist, British Petroleum Company, has been appointed as President-elect of the Royal Society of Chemistry at the Annual General Meeting of the Society.

Dr Richard P. Novick, a pioneer investigator in the field of plasmids and recombinant DNA (gene splicing), is the new Director of the Public Health Research Institute of the City of New York.

Ephraim P. Engleman, MD, a world authority on arthritis and its related diseases, has been elected president of the International League Against Rheumatism (ILAR). He is director of the Rosalind Russell Medical Research Center for Arthritis at UC-San Francisco, and will be the first US president in 30 years.

Dr Richard A. Anthes has been appointed director of the Atmospheric Analysis and Prediction Division of the National Center for Atmospheric Research (NCAR) in Boulder, Colorado.

Professor S. Desmond Smith, head of the Department of Physics at Heriot-Watt University since 1970, has been appointed Dean of the Faculty of Science in the University from 1 October 1981, for three years.

Meetings

12 October, **Mutagenicity Testing**, London (Mr M.L. Richardson, c/o Mr E.A. Taylor, The Royal Society of Chemistry, Burlington House, Piccadilly, London W1, UK).

18-23 October, **Liquid Scintillation Counting in Biology and Medicine**, Woods Hole (M.D. Maser, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA).

25-29 October, **Chlorinated Dioxins and Related Compounds**, Arlington (Dr R.E. Tucker, Dioxin International Symposium, PO Box 209, Rockville, Maryland 20850, USA).

27-28 October, **Moisture Measurements in Solids and Gases**, Chislehurst (Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

28-30 October, **Aging Brain and Ergot Alkaloids**, Rome (Dr A. Bosio, Dept of Pharmacology, University of Milan, Via Vanvitelli 32, 20129 Milano, Italy).

30 October - 3 November, **Critical Issues in Psychiatry for the 80's**, New York City (Meetings Management Dept, American Psychiatric Association, 1700 18th Street, NW Washington DC 20009, USA).

1-6 November, **Protein Analysis by Polyacrylamide Gel Electrophoresis**, Woods Hole, M.D. Maser, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA).

3-5 November, **Life Science '81**, New York City (D. Komar, Show Coordinator, 17 Washington Street, Norwalk, Connecticut 06901, USA).

4 November, **Animal Welfare in Agriculture**, London (Institute of Biology, 41 Queen's Gate, London SW7, UK).

9-11 November, **Microbiology and Toxicology**, Bristol (Society of Cosmetic Scientists, 56 Kingsway, London WC2, UK).

9-13 November, **International Scientific Forum on Changes in Energy**, Mexico City (University of Miami, Center for Theoretical Studies, PO Box 243055, Coral Gables, Florida 33124, USA).

10-13 November, **Magnetism and Magnetic Materials**, Atlanta (Dr H.C. Wolfe, American Institute of Physics, 335 East 45th Street, New York, New York 10017, USA).

15-20 November, **Biological Electron Microscopy for Technicians**, Woods Hole (M.D. Maser, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA).

16 November, **Environment of Venus**, Palo Alto (C. Redmond, NASA, Washington DC 20546, USA).

25-27 November, **The Chemistry of the Eighties**, Barcelona (2nd Mediterranean Congress of Chemical Engineering, EXPOQUIMIA-81, Plaza de Espana, Barcelona-4, Spain).

1 December, **Fume Cupboards, Fume Hoods and Ventilated Safety Enclosures for Laboratories**, Risley (The Symposium Organiser, Laboratory of the Government Chemist, Room 564A, Cornwall House, Stamford Street, London SE1, UK).

6–11 December, **Small Computers in Biomedical Research**, Woods Hole (M.D. Maser, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA).

15–17 December, **Pharmaceutical Technology and Product Manufacture**, Paris (Powder Advisory Centre, PO Box 78, London NW11, UK).

15–18 December, **Workshop for Biologists in the Water Industry**, Cardiff (Mrs P. Davies, WRC Process Engineering, Elder Way, Stevenage, Herts, UK).

6–7 January 1982, **Thermal and Mechanical Properties of Polymers**, Birmingham (Dr J.N. Hay, Dept of Chemistry, The University of Birmingham, PO Box 363, Birmingham, UK).

25 January — 5 February, **Immunochemistry Today**, London (School Office (SSC), Royal Postgraduate Medical School, Du Cane Road, London W1, UK).

9–11 February, **Non-invasive Studies of Body Chemistry**, Santa Monica (Dr W. Wolf, c/o Intrascience Research Foundation, PO Box 18589, Los Angeles, California 90018, USA).

15–17 February, **Hybridoma Research**, Los Angeles (Edward Ruffing, c/o Scherago Associates Inc., 1515 Broadway, New York, New York 10036, USA).

16–19 February, **Ocean Sciences**, San Antonio (American Geophysical Union, 2000 Florida Ave, NW Washington DC 20009, USA).

23–25 February, **Technical Aspects of Legal/Regulatory Pressures on the Pharmaceutical Industry**, Jerusalem (Powder Advisory Centre, PO Box 78, London NW11, UK).

7–13 March, **European Winter Conference on Brain Research**, French Alps (Dr S. Nicolaidis, College de France, 11 Place Marcelin-Berthelot, 75231 Paris Cedex 05, France).

25–27 March, **Neuroscience**, Valencia (Dr C. Guerri, Instituto de Investigaciones Citologicas de la Caja de Ahorros de Valencia, Amadeo de Saboya, 4, Valencia 10, Spain).

29 March–1 April, **Acoustics '82**, Surrey (Institute of Acoustics, 25 Chambers St, Edinburgh, UK).

30 March–1 April, **Dielectric Properties of Molecular Liquids and Biological Systems**, Cambridge (Dr C.W. Smith, The Dielectrics Society, Electrical Engineering Dept, University of Salford, Salford, UK).

30 March–2 April, **Annual Chemical Congress of the Royal Society of Chemistry**, Aston (Dr John F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).

14–15 April, **Risk Cost and Pollution**, Oxford (Mr L. Evans, Harwell Education and Training Centre, Harwell Laboratory, Oxon, UK).

25 April — 1 May, **EUCHEM Conference on Stereochemistry**, Burgenstock (Prof. Dr R. Huisgen, Institut für Organische Chemie, Universität München, Karlstr. 23, D-8000 München, FRG).

25–29 April, **Cell Function and Differentiation**, Athens (Special FEBS Meeting 1982, N.R.C. Demokritos, Dept of Biology, Aghia Paraskevi, Attikis, Greece).

26–28 April, **Application of Behavioural Pharmacology in Toxicology**, Capri (V. Cuomo, Institute of Pharmacology, University of Bari, Piazza G. Cesare Policlinico, 70124 Bari, Italy).

3–5 May, **Mining and Metallurgy Congress**, Johannesburg (Congress Manager, PO Box 809, Johannesburg, South Africa 2000).

4 May, **International Symposium on Crop Protection**, Gent (Secretary, Facultie van de Landbouwetenschappen, Coupure Links 533, B-9000 Gent, Belgium).

10–14 May, **52nd ANZAAS**, New South Wales (Prof. F.L. Milthorpe, Macquarie University, North Ryde, New South Wales 2113, Australia).

11–14 May, **Cryogenic Engineering Conference**, Japan (ICEC9–ICMC Secretariat, c/o Industrial Research Institute, Japan 2-1-7 Shinkawa, Chuo-ku, Tokyo 104, Japan).

18–21 May, **Prostaglandins**, Florence (Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23. 20121 Milan, Italy).

16–20 May, **Animal Counterparts of Human Disease**, Philadelphia (T. Mullarkey, Philadelphia, Pennsylvania 19104, USA).

24–28 May, **Immunocytochemistry and its Application in Brain Research**, Amsterdam (Dr F.W. Van Leeuwen, Netherlands Institute for Brain Research, IJdijk 28, 1095 KJ Amsterdam, The Netherlands).

6–11 June, **Synthesis and Applications of Isotopically Labeled Compounds**, Kansas City (Dr A. Susan, c/o Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, USA).

7–11 June, **Tunnelling '82**, Brighton (Institution of Mining and Metallurgy, 44 Portland Place, London W1, UK).

8–11 June, **Defined Immunofluorescence Immunoenzyme Studies and Related Labeling Techniques**, New York (Dr E. Beutner, State University of New York, School of Medicine, 219 Sherman Hall, Buffalo, New York 14214, USA).

14–18 June, **Reliability in Electrical and Electronic Components and Systems**, Copenhagen (The Conference Office, DIEU Danish Engineers', Post Graduate Institute, The Technical University of Denmark, Bldg 208, DK-2800 Lyngby, Denmark).

14–18 June, **Atherosclerosis**, Berlin (Prof. Dr G. Schlierf, c/o Medizinische Universitätsklinik, Bergheimer Str. 58, D6900 Heidelberg, FRG).

21–23 June, **Chromatography and Mass Spectrometry in Biomedical Sciences**, Bordighera (Dr A. Frigerio, c/o Istituto di

Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62-0157 Milan, Italy).

22–30 June, **Chemistry of Solid/Liquid Interfaces**, Dubrovnik (Dr V. Pravdic, Rudjer Boskovic Institute, PO Box 1016, YU 41001 Zagreb, Yugoslavia).

27 June — 2 July, **Darwin Centenary Conference**, Cambridge (Dr D.H. Mellor, Darwin College, Cambridge, UK).

28 June — 3 July, **Mathematics at the Service of Man**, Las Palmas (S.W.C. M, Ultramar Express S.A., Diputacion, 238, Barcelona-7, Spain).

28–30 June, **Pathological Anatomy and Cytology**, Copenhagen (NOPAC '82) Secretariat, Ibsttutterne, Frederik den V's veg, 2100 Copenhagen Ø, Denmark).

4–9 July, **General Relativity and Gravitation**, Venice (Prof. F. de Felice, GR10 — Istituto di Fisica "G. Galilei", University of Padua, Via Marzola, 8, 35100 Padova, Italy).

5–9 July, **Mechanisms of Reactions in Solution**, Canterbury (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).

6–9 July, **Inorganic Stereochemistry**, Reading (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).

12–14 July, **Chitin and Chitosan**, Sapporo (Dr S. Hirano, Dept of Agricultural Biochemistry, Tottori University, Tottori 680, Japan).

2–7 August, **IUPAC Symposium on the Chemistry of Natural Products**, Pretoria (CSIR Symposium Secretariat S. 219, PO Box 395, Pretoria 0001, Republic of South Africa).

2–13 August, **Joint Oceanographic Assembly**, Halifax (c/o Leo O'Quinn, 240 Sparks St, 7th Floor West, Ottawa, Ontario, Canada K1A 0E6).

9–22 August, **Methods in Computational Molecular Physics**, Bas Windsheim (Dr S. Wilson, Dept of Theoretical Chemistry, University of Oxford, Oxford, UK).

15–21 August, **12th International Congress of Biochemistry**, Perth (Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

15–22 August, **Generation of Major Basalt Types**, Reykjavik (Basalt Meeting, c/o Dr G.E. Sigvaldason, Nordic Volcanological Institute, 101 Reykjavik, Iceland).

30 August–3 September, **9th International Liquid Crystal Conference**, Krakow (Dr W. Witko, Institute of Nuclear Physics, 31-342 Krakow, Poland).

5–9 September, **Workshop on Drug Metabolism**, Liege (Dr J.E. Gilen, Institute of Pathology, Building B 23, Sart Tilman, 4000 Liege, Belgium).

5–10 September, **Invertebrate Pathology**, Brighton (H.D. Burges, Glasshouse Crops Research Institute, Littlehampton, Sussex, UK).

6–11 September, **Water and Ions in Biological Systems**, Bucharest (Prof. Dr. V. Vasilescu, c/o Union of Societies of Medical Sciences, str. Progresului no. 10, R-70754, Bucharest, Romania).